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On the Bionomics of the Vinegar Eelworm.

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Introduction.

From the previous paper on the anatomy of this worm (last volume, p. 183) may be recalled the fact that it is a free-living nematode, from 1 mm. to 2 mm. in length and about 0·04 mm. in breadth, which lives throughout its life-cycle in vinegar. As such, it clearly lends itself to biological research and has been used to this end by many workers and from different motives. Some have been merely curious about its habits, while others have regarded it as a possible internal parasite of man; in more recent years the question of its importance in the vinegar industry has arisen.

This paper is partly a review of the work of previous authors and partly an account of experiments by the present writer, and it will be convenient to treat the latter as a running commentary on the former rather than to separate them formally. Moreover, it is impracticable to keep the industrial, medical and purely biological aspects entirely distinct, though this will be done as far as possible.

The writer is indebted to Professor R. T. Leiper for his helpful advice, to Mr. J. Ramsbottom, O.B.E., of the British Museum, for information and references concerning the slime-fluxes of trees, and to the Manager and Works Chemist of a large vinegar factory in London for the courteous way in which they explained and demonstrated the process of manufacture and gave every facility for taking samples.

I.—Biological Observations.

Under this heading will be considered such physical factors as oxygen-deficiency, geotaxis, temperature, light and desiccation, and such chemical factors as acidity and the composition of culture-media.

A.—Oxygen-deficiency and Geotaxis.

As early as 1656 Petrus Borellus published a short note, "De Vermibus aceti," in his "Observationum Microcospicarum Centuria." He stated that the worms could be discerned in the neck of a flask of vinegar filled up with water, since they sought the surface of the liquid for the purpose of breathing. Goeze (1774), who carried out a series of biological experiments on the eelworm, maintained that they required air pre-eminently, that, therefore, they always tended to come to the surface of culture dishes, and that if the dishes were hermetically sealed the worms died. Lindner (1889) disagreed that air was the factor bringing them to the surface. "Their need for air and light is very small," he noted (p. 664), "since they thrive equally well in a tightly corked vessel filled completely and in one only half filled and left open or plugged with wool, and in a dark as well as in a light room. If a layer of oil an inch thick is poured on to vinegar containing eelworms, in a tube, the creatures go on living underneath the oil without being suffocated for want of air"—but he does not say for how long. Pallecchi (1893) boiled vinegar before introducing the worms, and in addition floated oil on the surface; most of these lived for thirty-six hours and some for several days, showing that the oxygen-need is slight. Henneberg (1900) agreed that the worms mostly died in about three days under completely air-tight conditions (*e.g.*, in an atmosphere of hydrogen); some, however, remained alive even after two weeks. "Thus they are less in need of air than would be concluded from their efforts to live

always at the surface of the fluid. The probable explanation of this is that they seek an opportunity of climbing out of the fluid. As soon as the vessel-walls are moist they leave the fluid and form a white slimy mass (resembling frost-spangles) above it."

The experiments of the present writer are in general agreement with those of Pallecchi and Henneberg, as the following results will show.

1. Paraffin Seal.—In a specimen-tube, a layer of purified (medicinal) paraffin, about 1 cm. thick, was floated on to vinegar containing a large number of eelworms. A few were still living at the end of fifty-five days. This experiment is not conclusive for the reason that as soon as the paraffin was added many of the eelworms passed into it, and throughout the course of the experiment the paraffin was more heavily populated with worms than the vinegar beneath. It was observed that the worms came to the surface of the paraffin, and even climbed well above it on the walls of the tube, afterwards returning to it. It is probable that at the paraffin-air surface there would be minute globules of moisture containing dissolved oxygen which would then become available for respiration (the worms appeared always to be separated from actual contact with the paraffin by a thin layer of vinegar). However, by the end of fifty-five days very few worms were living, while a control-tube (without paraffin) was swarming with them.

2. Olive Oil Seal.—Unfortunately, the worms behaved in the same indifferent manner towards olive oil. After twenty-two days in a culture sealed with it they were only slightly less active and numerous than in the control, and they swam about in the oil as freely as they had done in the paraffin.

3. Glass-sealed Tubes.—Test tubes were drawn out in the form of serum ampoules, infested vinegar was introduced so as practically to fill the tubes and the open ends were rapidly sealed in a flame. It was found necessary to support these tubes with the narrow end downwards, for in the reverse position the worms would crowd up into the narrowed neck to such an extent as to form a firmly wedged crust at the vinegar surface. The number of worms living in such a tube decreased

appreciably and their movements became more sluggish (as compared with an unsealed control) after five or six days; only a few remained alive after ten days, and by the end of four weeks they were all dead.

4. Microscopical Observations.—It was noticed that when a drop of infested vinegar was placed within an irregular ring of vaseline on a slide and covered with a slip, the worms quickly congregated at the vaseline wall and made every attempt to pass into it from the vinegar. This was particularly noticeable where the vaseline was indented to form narrow channels of vinegar; here, a dozen or more worms with their heads together would repeatedly rush into the channel and ram the vaseline. One or two managed to get through but most of them, after wandering for a while, returned to the vinegar.

In inoculating plates of gelatine with drops of infested vinegar, the worms quickly passed from the latter into the former, in which they continued to live and reproduce until the gelatine either hardened in some cases, or liquefied and became putrescent in others: a matter of about eight weeks in one instance.

5. The "Long-column" Tube.—An 85 cm. length of glass tubing, of 7 mm. internal diameter, was sealed at one end, and the other end, for a distance of 6 cm. or 7 cm., was bent through an angle of some 30° from the main axis. This latter arrangement allowed of the tube's being held horizontal, or slightly tilted sealed end uppermost, without any of the contained liquid's escaping.

Upon nearly filling the tube with infested vinegar and supporting it vertically with the sealed end down, the worms were seen to be markedly concentrated at the top within half an hour. The same was true when the tube was inverted vertically with the open end immersed in vinegar, although in this case there was no air at the top to attract them.

The worms having been concentrated at the open end by the method just described, the tube was now supported, sealed end uppermost, so that it made an angle of 15° with the horizontal; the bend at the lower (open) end prevented the vinegar from escaping. Within an hour there was a maximum concentration of worms at the upper end (where there was no air) and a smaller concentration at the lower open end (which was exposed to the air, and which was itself inclined upwards

at an angle of about 15°). The same upward movement was manifested when the tube was inclined at only 9° from the horizontal.

It is to be understood that in all cases a few worms were to be found in every part of the tube; the essential point is that they were present in greatly increased numbers in the upper few centimetres.

These experiments seem to show that the worms move upwards quite independently of any oxygen stimulus; the reaction may be described as a negative geotaxis, due either to gravitational "force" directly, or to fluid pressure.

6. Specific Gravity and Fluid Pressure.—It seems necessary to note at this juncture that the worms have a specific gravity greater than that of vinegar, so that their concentration at the upper layers of a liquid is due to their own efforts. Dead worms sink to the bottom, and in a centrifuge living worms are speedily brought to the bottom also.

It might be possible that the worms always move towards the region of least fluid pressure, however, or there might be an optimum fluid pressure (somewhere in the region of normal atmospheric pressure) below which the worms would cease to move upwards. Two experiments may be noted in this connection :—

(*a*) A test tube full of infested vinegar was placed in a glass vacuum jar (such as is used for storing soil samples), the mouth of which was guarded by an efficient valve. An exhausting pump was now connected and a negative pressure produced corresponding to about 700 mm. of mercury; this pressure was maintained by renewed pumping every few days. Incidentally the worms remained active and plentiful under these conditions for over 100 days; but the point of present interest is that they still congregated at the surface, in this respect not differing perceptibly from a control at ordinary pressure. Now 70 cm. of mercury is roughly equivalent in pressure to 1,000 cm. of water or vinegar, yet with this relatively great pressure removed, the worms continued to congregate at the surface of the liquid. It may therefore be concluded that there is no optimum pressure in the region of normal atmospheric pressure, and that if fluid pressure is the stimulus at all, then the worms tend to move towards the minimum pressure.

(*b*) A maximum amount of infested vinegar was supported by surface tension in a length of capillary tubing of 0·7 mm. bore, the tube being held vertically with both ends exposed; under these conditions the pressure due to gravity was exactly balanced by the surface tension. Within fifty seconds there was a maximum concentration of worms at the upper meniscus with about half the number at the lower. When the tube was held horizontally the worms quickly congregated at the two menisci. In both cases many of the worms were seen with their heads touching the meniscus and their bodies lashing vigorously, the appearance recalling their behaviour when passing from vinegar into vaseline.

7. *Worm-concentrations.*—In connection with the preceding experiments, estimations of the concentration of worms were made from time to time. Portions of 0·1 c.c. of infested vinegar were run from a micropipette on to glass slides, when the worms were killed by heat and counted. Ten such counts were taken as giving a rough estimate of the concentration. In most cases, the concentrations of worms in the samples used varied from 1,000 to 2,000 worms per cubic centimetre. At a fair approximation they would constitute about 1 per cent. of the total volume of such samples.

Discussion.—From the notes of previous workers such as Lindner, Pallecchi and Henneberg, and from the results of the experiments described above, it is clear that the amount of oxygen required by the vinegar eelworm is very slight, and is insufficient to explain the worm's constant efforts to reach the upper surface of the vinegar.

The determination of the actual oxygen requirement is clearly complicated by the presence of bacteria and fungi in the vinegar. It has not yet been attempted by the writer, since he has not yet grown aseptic cultures of the worm (*vide infra*, p. 21).

But there can be no doubt that the worms display a negative geotaxis which is independent of oxygen as a stimulus. Loeb (1918) has shown that the holothurian, *Cucumaria cucumis*, creeps up a vertical object until it reaches the highest level, where it remains, and that "Light and oxygen supply have nothing to do with the phenomenon" (p. 125). Loeb also mentions a similar behaviour in *Paramœcium*, and quotes Lyon to the effect that the body of *Paramœcium* contains substances

of different density which are so arranged that gravitation automatically maintains an upward orientation of the oral pole. It is doubtful whether the same mechanism is concerned in the case of the vinegar eelworm. In climbing the inclined tube, the movements of the worm are not regular. It will swim upwards for a couple of centimetres and then downwards again, and the normal orientation of the body appears to be horizontal. Neglecting the longitudinal distribution of worms in such an inclined tube, and considering their distribution in the plane at right angles to the length (the " transverse " distribution), it will be found that they are more abundant in the lower than in the upper half of the tube. A direct mechanical geotropism like that claimed for *Paramœcium* would require them to be concentrated at the top of the tube (considered transversely).

However, it may well be that the geotaxis is not directly due to the gravitational stimulus; the pressure of the fluid medium may rather be the factor; the worm may tend to move to the region where this fluid pressure is least. This would explain the upward tendency (on the average) of the worm and would be quite consistent with a general horizontal orientation and with the irregularity of progression up a tube (*e.g.*, the worm may be sensitive only to pressures of one or more centimetres of water).

The behaviour of the worm at the mensicus of vinegar in a capillary tube is very similar to that in narrow channels in vaseline or in the conical channel formed by sealing off a glass tube. The head of the worm remains more or less stationary at the apex of the channel and the whole body is lashed vigorously as if the worm were trying to penetrate the meniscus, vaseline or glass. The action resembles what Loeb (*op.cit.*) has called " stereotropism "—the tendency of an organism to bring its body as completely as possible into contact with solid bodies. The reaction normally takes the form of creeping into crevices, and is typical of earthworms and such creatures, which burrow into the ground. (In this connection, it may be pointed out that a liquid-air surface, approached from the liquid side, behaves much as a liquid-solid surface does. The writer has observed a planarian creeping, upside down and under water, across the water-air surface.)

The question of the purpose of these two reactions in the vinegar

eelworm must be reserved for subsequent consideration. It is clearly bound up with the question of the *natural* habitat of the worm, for the reactions are of little value to a worm living in a cask of vinegar.

B.—Temperature and Light Factors.

Goeze noted that by slightly heating the fluid in which vinegar eels were living the worms were quickly killed, while they were able to resist low temperatures well enough; even a brief period of freezing did not kill them. From this he concluded that, although perhaps able to resist the acidity of the human stomach, the worms would be killed by the heat of the body.

Lindner found that the temperatures most favourable to active reproduction ranged from 16° C. to 30° C. (temperatures will be recorded in degrees Centigrade throughout this paper). From 36° to 40° the mobility of the worms was increased for a time but soon diminished, and was lost altogether between 40° and 42°. At temperatures above 45° the worms quickly died. Temperatures below zero were equally fatal. Lindner's statement, that the worms thrive as well in the dark as in the light, has been noted above.

The findings of Pallecchi are more or less in agreement with those of Lindner. He found that the worms would live for one hour at 40°, while 45° killed them in from one to two minutes. Thus, he insisted, they could withstand the temperature of the human body. Of a culture of worms immersed in ice and salt at −20°, few survived; but they were indifferent to a temperature of zero and even to one of −5°.

Henneberg found the optimum temperature to lie between 20° and 29°. For the worms to remain living, the maximum temperature was 34° and the minimum 5°, but there was little or no reproduction under 14°; the worms were paralysed by heat at 40° and by cold at 3°. Temperatures from 42° to 43° killed them in five minutes, and 44° in one minute. They remained alive after 15 hours in frozen vinegar and lived for five hours at −20°. He found that they reacted to sudden intense illumination by increasing their mobility.

The temperature and light factors were investigated together by the present writer in an experiment involving four series of cultures, as follows :—

Series 1.—In daylight at room temperature.
Series 2.—In the dark at room temperature.
Series 3.—In the dark at 0·5°.
Series 4.—In the dark at 37°.

Each series consisted of duplicates and an uninfested control. Each culture contained 5 c.c. of vinegar, the volume being maintained with distilled water. The concentration of worms was about 1,000 per c.c. The results were as follow :—

Series 1 and 2.—At the end of 46 days there was no perceptible difference between these two series. Worms were living actively and at concentrations comparable with that at which the experiment started. The presence of many young forms testified to the fact that reproduction still proceeded. There was a profuse growth of a slimy fungus.

Series 3.—At the end of 46 days the worms were about half as plentiful, and not quite as active, as they were at the start. Here again many young were present, which fact is at variance with Henneberg's conclusion. Neither slimy fungus nor putrid smell was perceptible.

Series 4.—In this case there was a marked reduction in numbers, though not in activity, by the end of the fourth day. After 9 days all worms were dead. One of the duplicates had become very opaque.

An unsuccessful attempt was made to adapt the " long-column " tube for testing the reaction of the worm to temperature. The tube was supported slightly out of the horizontal with the lower (closed) end immersed in ice and water and the upper end maintained at a temperature of 70°. Although a shorter tube was used than previously (effective length = 35 cm.), the change in temperature along the tube was too far from uniform for the results to be of any value.

As regards the effect of light, the " long-column " tube (as described in the experiments on geotaxis) was supported horizontally with one half darkened and the other exposed to daylight, but no perceptible reaction was given. Again, light from an electric filament was passed through a water-bath and brought to a sharp focus successively at different points along a horizontal capillary tube containing infested vinegar, but the normal distribution of the worms was not affected.

The results obtained by Lindner, Pallecchi and Henneberg are in substantial agreement with one another and with those of the present writer. Briefly, the worm will live and reproduce at temperatures varying from zero to 35°. Temperatures of −20° on the one hand and 45° on the other are quickly fatal. The worms are practically indifferent to the influence of light.

C.—Desiccation.

Linnæus gave the specific name *redivivum* to the worms from vinegar and from bookbinders' paste, apparently because they were thought to be capable of reviving after desiccation. In fact, he used the word (adjectivally) in his description of the genus *Chaos* as a whole. O. F. Müller (1783) quoted Ledermüller as having asserted the same, but himself disagreed with Ledermüller on experimental grounds. "Nothing that is truly dead can revive (without a miracle)," he explained—which is scarcely the point at issue. Goeze (1774) held that at least the eggs could withstand desiccation, and that the worms were spread in fact by the eggs being blown about as dust. Hogg (1863) was of the same opinion. Lindner and Henneberg, however, agreed that the worms would not revive after drying.

Goeze claimed that the worms were oviparous in winter and viviparous in summer. The writer has not been able to find free eggs at any time of the year: his observations are that the egg-membrane is burst just before or during the process of birth. In this connection Henneberg states that "The young worms are born in an egg-membrane which immediately bursts." In any case, this membrane is very thin and frail, contrasting sharply with those nematode eggs (*Ascaris*, *Trichocephalus*) which *are* adapted to withstanding more or less complete desiccation.

A drop of vinegar was allowed to evaporate slowly from a glass slide until the remaining smear was just tacky. At this stage, at which the worms were seen to be quite motionless, the slide was immersed in vinegar. Close observation for a week failed to reveal the least sign of life either in the adult worms or in the embryos *in utero*: the test was repeated several times with the same negative result. Under these conditions the acetic acid and other substances dissolved in the vinegar become

increasingly concentrated as the liquid evaporates; thus the worms might be killed by the excessive concentration of dissolved substances rather than by the physical fact of desiccation. To test this point the worms were brought (by filtration) into distilled water, and drops of infested water were allowed to evaporate from slides. The results of these tests showed that the worms would revive after complete desiccation, but only when desiccation had been maintained for not more than ten minutes.

D.—The Influence of Acidity on the Eelworms.

Goeze noticed that the eelworms quickly died in mineral acids. This was confirmed by Davaine (1865), who found in addition that "They could not live very long in oxalic, citric and acetic acids diluted with pure water to the acidity of vinegar, but they lived and propagated rapidly in non-acid fluids which contained sugar." Goeze said that they lived for a time, but did *not* propagate, in sugar solutions. The explanation of this anomaly is probably to be found in Davaine's observation that the worms were quickly killed by the rapid production of lactic acid in sugar solutions: he overcame this difficulty by adding to the culture a layer of pulverized chalk which neutralized the lactic acid; after this the worms lived normally.

Hogg mentioned that eelworms were "only found in the inferior and diluted vinegars of commerce"; they never occurred in stronger superior vinegars because "care is taken to separate all mucilaginous or albuminous matters from the vinegar." This was effected by refining processes and "by the addition, allowed by law, of a certain quantity of oil of vitriol."

Lindner could find few or no eelworms in vinegars of the strength directed by the "Pharmacopœa Germanica" (6 per cent.; 7 gm. should saturate 1 gm. of hydrated sodium carbonate), but they were plentiful in the much more dilute vinegars sold in shops. Pallecchi, on the contrary, found them in Italy more plentiful in the stronger samples. They were able to live in 15 per cent. acetic acid, and they retained their full vitality and reproduced actively in 10 per cent. In wine vinegar Lindner very rarely found any worms, and then only isolated individuals: according to Pallecchi they were almost always to be

found in it, in more or less large numbers. Henneberg, in closer agreement with Lindner, found that 15 per cent. acetic acid was fatal to the worms in five hours, and that they could live only six weeks in vinegar containing 12 per cent. acetic acid. " In 10 per cent. vinegar they can live, but reproduction is very slow; considerable multiplication takes place only in vinegars with an acidity of under 6 per cent. Indeed, the less acid the vinegar, the more suitable it is " (Henneberg, *op. cit.*).

These results are very conflicting, but a possible clue is given in Hogg's note, that worms are not found in strong vinegars to which sulphuric acid has been added. All the experiments of the workers mentioned above were carried out with solutions standardized as *percentage* concentration of acid, whereas a standard of chemically Normal concentrations would have been preferable for comparative purposes. For instance, Pallecchi compared the times required by the chief mineral acids to kill the worms, but as the acids were all of 5 per cent. strength the results are not comparable. Even the adoption of normality standards is insufficient for any but the strongest acids, since it is now realized that the total concentration of acid, as determined by titration, does not represent the true acidity factor of a biological environment. In terms of the Dissociation Theory (which is sufficiently trustworthy for present purposes) the molecules of an acid are dissociated to a greater or less extent (according as the acid is more or less strong) into acidic ions and hydrogen ions. Thus, in a Normal solution of HCl, practically all the molecules are so dissociated, while in a Normal solution of acetic acid a considerable proportion of the molecules remains in the molecular (undissociated) condition. Moreover, it is the hydrogen ions which give to the solution its acidic properties; so that the concentration of hydrogen ions, and not the total concentration of acid, is the important factor. In illustration of this fact it may be noted that vinegar eelworms thrive in a normal solution of acetic acid (which is roughly 6 per cent.), while they die in a few hours in a 0·1 normal solution of hydrochloric acid (roughly 0·4 per cent. HCl), and in a few seconds in a Normal solution.

Thus the addition of small quantities of sulphuric acid to vinegar would result in a considerable increase in the hydrogen ion concentration. Interesting data on this point were provided by Kling,

Lassieur et Lassieur (1922) in a paper on the determination of hydrogen ion concentrations as applied to the examination of mineral acids in vinegar. Using the electrometric technique with a hydrogen electrode, they determined the hydrogen ion concentrations of various samples of wine and spirit vinegar, before and after the addition of sulphuric acid to N/20 strength ; the total acidity was also determined. From averages of eight samples the results were :—

Acidity as acetic acid per cent. ... = 6·2.
pH before addition of H_2SO_4 at 18° = 2·71.
pH after addition of H_2SO_4 to N/20 = 1·70.

(pH is the symbol for the logarithm of the reciprocal of the hydrogen ion concentration expressed in grammes per litre ; it is known as the hydrogen ion " Exponent," and it clearly varies inversely as the acidity. pH : 0 represents the Exponent of a Normal solution of hydrogen ions, pH : 7 represents " neutrality " [at about 25°,] pH : 14 represents the Exponent of a Normal solution of hydroxyl ions.)

These averaged results show that a small quantity of sulphuric acid is capable of raising the hydrogen ion concentration considerably. But examination of individual results reveals a more interesting point ; the Exponents for the untreated samples are not regularly proportional either to the total acidity or to the Exponents of the samples after the addition of constant quantities of sulphuric acid. In other words, samples of the same total acidity give different pH values, and samples of the same pH value give different pH values after the addition of sulphuric acid. This result is probably due to the presence, in varying proportions in the different samples, of electrolytes other than acetic acid ; these modify the hydrogen ion concentration—displaying what is termed a " buffer " action.

The upshot of this is that the only valid way of investigating an organism's response to acidity is to standardize the acid in terms of hydrogen ion concentration—or preferably in terms of its Exponent, for in most biological processes the relation is logarithmic rather than direct.

An attempt was made by the writer to determine the lower pH limit for the worms. This was done by culturing them in buffer solutions—solutions of electrolytes having a known and fairly stable

Exponent. A vinegar culture was filtered and the worms washed repeatedly on the paper with distilled water, into which they were finally transferred; this is referred to briefly as "infested water." The following cultures were then prepared in small Petri dishes:—

1. 10 c.c. 0·2N HCl, + 10 c.c. infested water; pH : 1·0 (Sørensen).
2. 5 c.c. 0·2N KCl, + 4·15 c.c. 0·2N. HCl, + 10 c.c. infested water, + distilled water to 20 c.c.; pH : 1·4 (Clark & Lubs).
3. 5 c.c. 0·2N KCl, + 1·66 c.c. 0·2N HC1, + 10 c.c. infested water, + distilled water to 20 c.c.; pH : 1·8 (Clark & Lubs).
4. 5 c.c. 0·2N KCl, + 10 c.c. infested water, + distilled water to 20 c.c. (First Control).
5. 10 c.c. infested water + distilled water to 20 c.c. (Second Control).

It will be seen that the same concentration of worms occurs throughout, and the same concentration of KCl in *2*, *3* and *4*. These are all unnatural media for the eelworm: if they died in the first three cultures, death might be due to the chlorine ions present, or to the potassium ions in *2* and *3*: the fourth culture was intended to control this factor. Finally, if they died in the fourth culture, death might be due to sudden removal from vinegar to such an unnatural medium as distilled water: the fifth culture controlled this factor.

The results may be summarized as follows (the percentages are rough estimations):—

After 3 hours—

1. 50 per cent. dead; remainder writhing at bottom of dish.
2. 20 per cent. writhing at bottom; remainder active.
3. 5 per cent. writhing at bottom; remainder active.
4 and 5. As 3.

After 6 hours—

1. With a single exception, all dead.
2. 10 per cent. dead; 70 per cent. writhing; remainder active.
3. 5 per cent. dead; 20 per cent. writhing; remainder active.
4 and 5. 5 per cent. writhing; remainder active.

After 18 hours—

1. All dead.

2. 80 per cent. dead ; 15 per cent. writhing ; a few active.

3, 4 and 5. As after six hours.

After 300 hours.

2. All dead.

3 and 4. Majority still active.

5. Majority dead.

Thus it may be concluded that media with a pH value of 1·4 or less are fatal to the worm : as a rough practical guide, any medium which presents with the indicator Thymol Blue (thymol-sulphonephthalein) the full red " virage " is too acid for the culture of the worm.

Returning to the consideration of previous work on this acidity question, it is clear that discrepancies can arise in observing the worm's toleration for vinegars of differing " strength " (where this implies percentage of total acid), even assuming that this toleration is a constant factor for all cultures of the worm. For, in titrating vinegar, the total acid is measured as if it were acetic acid. Thus Kling, Lassieur et Lassieur record a sample with a total acidity of 6·15 per cent., and with a pH value of 2·68 : eelworms would readily live in such a sample. The addition to this sample of sulphuric acid to only 0·24 per cent. depressed the pH value to 1·43, and in such a sample the worms would almost certainly fail to survive. Yet the presence of the sulphuric acid would not be revealed in titration : it would be included, as a very small proportion, in the percentage of " acetic acid."

The lower limit of hydrogen ion concentration has not received much attention from the previously mentioned workers. Most of them are content to say that the worm will survive in acid or neutral, but not in alkaline media. The writer sought to test this point, employing exactly the same technique as is described above, but using Clark and Lubs' " Boric acid and KCl—Sodium hydrate " buffer mixtures. The Highest exponent conveniently stabilized by this buffer is pH : 10·0. In a culture with this Exponent a few worms were still living after 48 hours, and, since the technique is reliable only for short-period reactions, buffer mixtures covering a more alkaline range were desirable. For this purpose Sørensen's " Glycine and NaCl—NaOH " mixtures were

used, with controls to test the separate chemical effects of glycine, sodium chloride and sodium hydrate.

It was found that O·1N sodium hydrate was fatal within a minute (pH: 13 approx.). Glycine of the maximum strength (0·1 Normal) also proved fatal after a few hours, thus severely limiting the time-limit of the experiment. Nevertheless, well within this limit there were marked differences in the reactions of the worms to the various solutions. Thus after one hour, the great majority of the worms were: (*a*) dead or dying in solutions of pH. 12·0 and over, and (*b*) normally active in solutions of pH: 11·5 and under. These results suggest that the lower toleration limit of hydrogen ion concentration (*i.e.*, the upper pH-limit) lies in the region of pH: 11·5, which, to say the least, is distinctly alkaline.

The upper and lower limits are largely masked in the above experiments by chemical influences other than acidity. Moreover, at the best they apply to the worm's capacity for remaining alive: in their nature, such experiments could not be extended long enough to determine the toleration limits for reproduction. But they at least reveal the remarkable resistance offered by the worm to media as acid as pH: 1·8 and as alkaline as pH: 11·5. If more extensive data are ever required (as for present purposes they are not) the way is clear for the use of buffered culture media such as are employed in bacteriology.

E.—The Influence of the Eelworms on Acidity.

The hydrogen ion concentration of a liquid in which animal organisms are living tends to be increased owing to the production of CO_2 in respiration and its solution as carbonic acid (in plants the process is complicated by the absorption of CO_2 in photosynthesis). This effect is usually only temporary (unless, as in alkaline solutions, the CO_2 is fixed as a carbonate), since the CO_2 in solution attains to equilibrium with that in the surrounding air. If the CO_2 be driven off (*e.g.*, by efficient aeration), then any residual change in hydrogen ion concentration will be due to factors other than CO_2—or at least to factors other than volatile substances, of which latter CO_2 is likely to be the most important. In the case of organisms that will live in indicator solutions a technique is thus suggested for determining the respiration-rate, which may be

regarded as a function of the rate of metabolism, as well as for investigating the immediate effect of the organisms on the acidity of the medium. Previous work on aquatic planarians and leeches (unpublished) has shown, however, that many difficulties arise in applying such a technique. Different indicators used with the same organism under parallel conditions require different times in which to attain to the same result; there is a differential adsorption by the tissues of the organism of different indicators; even the useful layer of paraffin (used as a seal to prevent the interchange of gases) will adsorb certain indicators (Neutral Red: red colour-form absorbed from yellow solutions; Methyl Red: yellow colour-form adsorbed from red solutions). Moreover, in the case of the vinegar eelworm the medium is complicated by the presence of other organisms—bacteria and fungi. If the experiment were conducted with the latter present they would contribute to the final effect to an unknown extent: if pure cultures of the worm were used, this would not represent the true effect under normal conditions. Accordingly, a compromise was reached in the following way.

Vinegar eelworms together with the other organisms were cultured in a solution of the indicator Bromo-cresol Green in tap water: the solution was adjusted with acetic acid to pH: 4·5, which is approximately the mid-point of the range of the indicator, and was sealed with a layer of liquid paraffin. The tap water and acetic acid yield a slightly buffered solution: theoretically, distilled water would be more sensitive to the change, but in practice a feeble buffer action is useful in counteracting the glass-alkali influence which quickly manifests itself even after a thorough cleaning of glassware. A second culture was prepared from which the eelworm, but not the other organisms, was absent: apart from this difference the second culture was identical with the first. Third and fourth cultures without the paraffin seal were otherwise identical with the first and second respectively; in the latter pair any volatile substances such as CO_2 were free to escape. Theoretically the following results should be deducible from the effects observed:—

1=Total effect of all organisms on acidity.
3=Effect of non-volatile products of all organisms.
1–3=Effect of volatile products of all organisms.

2=Total effect of organisms other than eelworms.
4=Effect of non-volatile products of organisms other than eelworms
2–4=Effect of volatile products of organisms other than eelworms.
1–2=Total effect of eelworms alone.
3–4=Effect of non-volatile products of eelworms alone.
(1–2)—(3–4)=Effect of volatile products of eelworms alone.

Actually, of course, this ideal analysis is not realizable. For one thing, the behaviour of " other organisms " in the presence of eelworms is not comparable with their behaviour in the absence of eelworms, since the two groups influence each other in various ways (*e.g.*, the eelworm feeds on the bacteria : see below) ; in other words, the various " factors " of the analysis are not independent one of another, and so cannot be treated as algebraic functions. Nevertheless, the results of the experiment are not without interest.

The estimated concentration of worms was 970 per cubic centimetre and the volume of each culture was 10 c.c. No colour-change was perceptible until after the fourth day ; by the sixth day 2 and 4 (without eelworms) were slightly more alkaline, while 1 and 3 were still unchanged. By the fourteenth day 2 and 4 had become markedly alkaline, while 1 and 3 had become perceptibly more acid. At the end of a month the worms were still living actively in 1 and 3, and the virage represented a pH value of 3·8 (practically at the acid end of the range of the indicator) ; while in 2 and 4 was seen an extensive growth of a dendritic fungus at the bottom of the culture, and the virage had passed to the alkaline end of the indicator's range (pH : 5·2 or above). The unexpected result was that there was no perceptible difference between the virages of 1 and 3 or between those of 2 and 4, even after 3 and 4 had been aerated. No valid quantitative results are deducible, but qualitatively the experiment seems to show that the eelworms have an acidifying effect on the medium in which they live, the effect being permanent and therefore probably not due to the CO_2 of respiration. Conversely the bacteria and fungi appear to make the medium more alkaline, though a control suggested that this effect was augmented by a slight glass-influence. Although the change in the hydrogen ion Exponent was considerable (about pH : 0·8 in each direction within a month), yet subsequent

titration showed that the change in total acidity was extremely slight in both cases ; the change in Exponent would be considerably less in a more perfectly buffered medium like commercial vinegar.

F.—Chemical Factors other than Acidity.

The vinegar eelworm is extraordinarily resistant to a number of chemical substances which quickly prove fatal to most other organisms. Pallecchi is the authority on this subject, for he listed several dozen different reagents, with notes on the behaviour of the worms in each case. No good purpose would be served by quoting his lists in full, but it is interesting to find that worms lived three days in 1 per cent. chromic acid, indefinitely in a saturated solution of tannic acid, four days in 2 per cent. potassium dichromate, and over eighteen hours in 1 per cent. solutions of morphia, atropine and strychnine. Fifteen per cent. alcohol afforded quite a good culture medium. Among the common salts, it appears from Pallecchi's results that the sulphate ion is relatively very toxic.

It is quite clear from Pallecchi's article that his chief concern was to show that the worms could live in the human body : thus he recorded that they would live in human saliva, in pepsin, gastric juice, fowl and ox bile, and in human fæces and urine.

Apart from experiments with acetic and mineral acids and with alcohol, Henneberg was mostly concerned with providing suitable culture media—a subject to be considered in the succeeding section.

In the course of anatomical and biological work on this worm the writer has made some miscellaneous observations which may conveniently be considered here. The worms are extremely sensitive to iodine, as, indeed, previous writers have noted, and to ammonia : a hanging-drop culture, for instance, is killed in a few seconds by the ammonia escaping from a bottle of strong ammonium hydrate. Chloroform too is quickly fatal to them ; they were killed in 40 minutes by a 10 per cent. solution of chloroform water (containing about 0·04 per cent. chloroform). On the other hand they lived over 30 hours in dilute vinegar with a layer of ether floating on it—many in fact passed into the ether layer, returning to the vinegar after several seconds. Their capacity for living in the indicators and buffer solutions used in hydrogen ion determinations has

already been noted : in this connection, Walpole's " Acetic acid—Sodium acetate " solutions (range, pH : 3·6–5·6) are very suitable media. Of course, they will not live indefinitely in sterile culture in buffer solutions, for apart from the absence of food many ions (such as Na^+, K^+, HSO_4^-, Cl^-, etc.) are toxic after a short time. Little harm, however, results from indicators and intra-vitam stains, as has been recorded in the writer's previous paper on the anatomy of the worm.

G.—Culture Media.

A large range of media has been successfully used by various workers : for instance, most of the bacteriological media are suitable (Henneberg). Davaine found that the worms lived several months in a 0·4 per cent. solution of sugar in pure water. Guided by this result, he tried culturing them in a large variety of fruits and vegetables with considerable success. The number of worms resulting from reproduction in such a medium always seemed to depend, he said, on the quantity of sugar present. They were also cultured easily in flour-paste. Lindner also found flour-paste and fruits to be good media ; the fruits, however, " rapidly become decomposed by the continued presence of the worms in them." His gelatine culture is very useful : not only are the movements of the worm slowed down so as to be easily observable, but (if the gelatine be sufficiently viscous) the progeny of each female remains in her neighbourhood and in this way are formed colonies lending themselves to statistical studies. The present writer has found 9 per cent. gelatine in a mixture of equal parts of vinegar and water to be a suitable proportion. Lindner also successfully used egg albumen acidified with vinegar. Henneberg noted that the worms thrive in a large variety of media, " But they die as soon as putrefaction sets in." He accordingly recommended that the cultures should contain 3 per cent. acetic acid or 12 per cent. alcohol in order to exclude putrefactive bacteria. Meat-, fruit- and malt-extracts all proved very suitable. Henneberg claimed that bacteria and starch-granules form the food of the worm : he demonstrated the presence of both in the worm's intestine, and successfully reared the worms in a culture of the hay bacillus ; yeast-cells, however, were found to be too large to pass through the narrow œsophagus. In all cases the media might be acid or neutral, but not alkaline (putrid)—[" . . . aber nicht alkalisch (faulig) "]. It

seems doubtful whether alkalinity as such is really the limiting factor (see section on acidity above): more probably the latter is a toxic substance (ammonia ?) produced by the protein-decomposition of which alkalinity is merely the sign. The writer kept one culture in vinegar, to which large quantities of neutral red had been added, for some seventy days. At the end of this time the culture was giving off a most offensive putrid smell, and its hydrogen ion Exponent had risen from pH : 2·6 to pH : 8·0. Although eelworms were living under these conditions, they had fallen off considerably in numbers, yet media of pH : 8·0 are decidedly not toxic as regards alkalinity alone.

Henneberg's claim that the worms actually feed on bacteria is, if true, of no slight importance to the vinegar industry. Moreover, such a mode of nutrition might be found to apply to other free-living nematodes, or even to plant-parasitic forms, in which case considerable light would be shed on a number of problems now obscure. Significant, therefore, is the interesting work of Zimmermann (1921) on the aseptic culture of the vinegar eelworm. Basing his work on the successful aseptic culture of *Drosophila ampelophila* by Guyénot in 1917, Zimmermann subjected eelworms to the antiseptic action of oxygenated water for ten-minute intervals twice a day over a period of ten days: between these intervals the worms were immersed in sterile vinegar. This prolonged sterilization was necessary to ensure that all bacteria passed from the worm's intestine; moreover it was quite satisfactory, for culture media inoculated with the worms remained sterile at all temperatures. The worms were now introduced into various media. In *fresh* sterile potato-starch paste, as in sterile and *filtered* vinegar, they lived only a few days. In *fermented* paste which had been subsequently sterilized, and in sterilized extract of mother of vinegar (*i.e.*, which contained the dead bodies of bacteria), the worms lived and reproduced actively. Thus, Zimmermann concluded, the worms feed upon the bacteria themselves (dead or alive) rather than upon the fluid medium. He afterwards succeeded in culturing the worms in an artificial medium. The basis of the latter was peptone, lecithin and mineral salts, but this was insufficient without the presence of an unknown substance derived from the autolysis of yeast. But the point to be emphasized here is that, under natural conditions, the worms feed on bacteria (as Henneberg claimed); there can be little doubt

that the living ferment, *Mycoderma aceti*, which is essential to the manufacture of vinegar, serves as a source of food to the vinegar eelworm.

In the extensive literature which has appeared on this worm there are considerable discrepancies in the values given for its anatomical dimensions : further, the worm is variously described as being oviparous or viviparous. These anatomical points have been dealt with elsewhere but it is pertinent to note here a statement by Henneberg. He said : " The size of the eelworms varies according to their food. The smallest are found in vinegar made by the Rapid process, the largest in liquids rich in bacteria and organic matter. Shorter and broader forms are to be seen in acid cultures of barley-malt." He also notes that a female worm may die before all the young are hatched, in which case the latter may be found wandering about inside the dead body of the mother. Regarding these points, the work of Conte (1900a and 1900b) on *Rhabditis monhystera* is of importance. He found (1900a) that the medium in which the worms were cultured exercised a decided influence on the dimensions, and on the conditions of development, of the worm. Thus in flour-paste the worms were viviparous : one female contained 105 eggs and twenty developing embryos. In peptone solutions they were oviparous, some eggs being laid even at the two-cell stage, and the number of eggs *in utero* at any one time was reduced to as few as six or seven. The size of the worms varied with the richness of the medium : after four months' culture in paste the length was reduced by more than one-half (of two cultures of vinegar eelworms studied by de Man (1910) the dimensions of the worms in one were about a half of those of the worms in the other). With reference to the conditions of birth, Conte (1900b) observed that the eggs of *Diplogaster longicauda* (normally oviparous) hatched *in utero* when the medium became putrid. He saw female *Rhabditis* worms being devoured by their own offspring which had hatched *in utero* and had perforated the uterine wall. Conte recognized various degrees from oviparity, through viviparity, to larval parasitism, and correlated the latter with a morbid state of the mother—probably due to putrefaction of the medium (rather than to inanition and senility, as Maupas had suggested).

These facts have an obvious bearing on questions of anatomical description and even of systematics, but they are mentioned here in

support and in extension of Henneberg's observation—that the dimensions of the vinegar eelworm are partly determined by the nature of the medium in which they are living.

In his anatomical paper the writer has mentioned the numerous refractive globules which surround the intestine of the worm, and it is there shown—from the results of staining with Scharlach R and Nile Blue—that these globules consist of neutral fats. After periods of starvation they are greatly reduced in number, and it is evident that they are reserve food-materials. References to them have been subsequently found in the literature. Dujardin (1845), in parenthesis, refers to them collectively as the liver. Henneberg considers them to be of a fatty nature, and adds that glycogen is regularly present, probably also as a reserve food-material. When the worms die (he notes) the globules run together into two or three large globules which form after a time the sole contents of the thin skin (the present writer has often found masses of dead worms in this condition : they resemble chains of giant yeast-cells). It is of interest to note that Conte (1900a) records a similar production of " reserve granulations " in *Rhabditis monhystera ;* there, " They invade the intestinal epithelium, then the hypoderm, until the animal is quite opaque " : in worms living in an exhausted medium the granulations are less abundant and are often localized in the uterus, where their accumulation often coincides with a temporary sterility due to atrophy of the ovary. The writer has never found fat globules closely associated with the uterus in the vinegar eelworm.

This synthesis of relatively large proportions of neutral fat from the substances present in vinegar is very remarkable and might offer interesting material to the biochemist.

II.—The Question of Pathogenicity.

In the brief description of the vinegar eelworm given by Borellus occurs the remark that many people ceased to use vinegar after he had demonstrated the worms to them : Borellus himself offers no comment on this cautious attitude. But from the seventeenth century, when he wrote, down to the present day there have appeared elaborate arguments intended to prove or disprove that the worm is a human parasite. Clearly the worm is not an obligatory parasite, since it will live for an indefinite

number of generations in vinegar ; but it might be a facultative parasite adapting itself to the conditions of existence in the human body.

It will be recalled that Goeze rejected this possibility on the grounds that the temperature of the human body would be fatal to the worms, but this was only a rough guess : accurate thermometers did not form part of the equipment of this early biologist.

It is true that, according to Jabez Hogg, the presence of these worms in the human body was " demonstrated " by the quacks of his time, but only in a highly regrettable manner. The unfortunate patient was invited to see, through a microscope, vinegar or paste eels swarming in " A drop of fluid derived from his natural juices " : the slide had been smeared with a culture of the worms just previous to the consultation !

Oerley (1886) mentioned the vinegar eelworm in his essay " Über die Rhabditiden und ihre medicinische Bedeutung," but only to emphasize its harmlessness to man (*fide* Lindner). Lindner himself was more cautious. Having shown that the worms could live in the virtual absence of air and light, and could withstand body temperature and the acidity of gastric juice, he pointed out that they might be able to live as parasites for a considerable time under favourable conditions. Indeed, he quoted the clinical evidence given by Wiel in his " Diätetischen Kochbuch für Gesunde und Kranke " (1881, p. 178). All the members of a household in Wiel's medical practice had for some time suffered from a gastric complaint. On inspecting the kitchen stores he found some vinegar in which there was a veritable ball of living eelworms : seen through a hand-lens the vinegar was swarming throughout with them. The removal of this infested vinegar coincided with the rapid disappearance of the symptoms. Lindner also fed the worms to a mouse, and found *post mortem* that many worms remained living in the cardiac region of the stomach ; many were dead near the pylorus, and there were more dead than alive in the duodenum and jejunum. Bile, he concluded, was fatal to them. This involves no contradiction of Wiel's evidence, for in his case the symptoms were purely gastric, and worms were being continuously introduced to take the place of those which passed from the stomach.

An account has already been given of the work of Pallecchi. He persuaded the eelworms to live in a large variety of body fluids and at

body temperature, and seemed convinced of their pathogenic importance.

In 1898 the political Press in Chile reported the occurrence of worms in vinegar and made the unhappy error of naming them *Anguillula stercoralis* (a synonym of *Strongyloides stercoralis*). Accordingly, del Rio (1898) corrected the error and made some observations on the significance of the worm to public health. After summarizing Lindner's work and calling attention to the case of Wiel, he concluded that infested vinegar should not be consumed for the following reasons: (*a*) The presence of the worm indicated that vinegar had been manufactured under conditions of doubtful cleanliness; (*b*) it also indicated that the vinegar was poor in quality (*i.e.*, containing less than 4 per cent. acetic acid); and (*c*) finally, to consume such worms was repugnant. Heating to a temperature of 50° or treatment with sulphuric acid, with subsequent filtration in either case, was suggested as a remedy. The question of pathogenicity del Rio left open.

Henneberg noted that the worms could live for several hours at 38°–39°, but (he said) they were so weakened by the high temperature that they could not occasion any trouble in the body, and would in any case be killed by the alkaline reaction of the intestine. Further, had the worms been pathogenic this fact would have been discovered, since they must frequently have been introduced into the human body (he had apparently not heard of Wiel's case). He found that, fed to a frog, the worms passed unharmed through its intestine, but he attributed this to the low body-temperature of the amphibian.

Discussion.—From a consideration of the effects of heat on the worms, it is probable that they could withstand body temperature for a few days, but at the same time it is highly improbable that they would reproduce and so maintain their existence. In the writer's experiments a temperature of 37° was fatal to all worms in nine days, yet the young normally attain sexual maturity only after four weeks (Henneberg).

The questions of the acidity and alkalinity of the stomach and intestine must necessarily be reconsidered from the point of view of hydrogen ion concentrations. In this matter, more or less reliable determinations have been made by various workers (see the extensive bibliography in Clark, W. M., "The Determination of Hydrogen Ions," 2nd Edition,

1922). The following values of the hydrogen ion Exponent for various body fluids are given in Clark and Lubs (1917), pp. 219-220 (N.B.—"Neutrality" at 37° = pH : 6·8, instead of pH : 7·0).

Body fluid.	pH.
Saliva	6·9
Gastric Juice (Adult)	0·9–1·6
Contents of small intestine	8·3
Bile (from gall-bladder)	5·3–7·4
Fæces	7·1–8·8

Comparing these values with the toleration limits deduced by the writer and with the theoretical arguments put forward by previous authors, it will be seen that the conclusions of the latter will have to be reversed. From the point of view of alkalinity the contents of the small intestine would be harmless—the bile is sometimes distinctly acid; and the acidity of the stomach, which (all agreed) the worms would easily resist, is seen to be in the neighbourhood of the lethal limit—certainly a pH of 0·9 would very quickly be fatal.

Chemical influences other than acidity are, of course, another matter, and concerning these there are only the experiments of Pallecchi—apparently performed at room temperature.

Interesting as may be the theoretical arguments for and against a facultative parasitization of the human body by vinegar eelworms, they yet remain inconclusive and in many respects conflicting. The writer therefore decided to make a more definite test. Accordingly, 20 c.c. of a vigorous culture were diluted, sweetened and drunk, the dilution and sweetening factors being observed in a duplicate control. The number of worms so consumed, as estimated by counts of the culture, was about 36,000. Two days later a second and equal quantity was drunk. Fæcal cultures in vinegar were prepared each day for five days, and again after forty-seven days, but in these, as in fæcal smears, there was no sign of worms either living or dead. Throughout the experiment there were no perceptible symptoms whatever.

This experiment *proves* merely that no observable symptoms arose in one particular attempted infection, but it does also *suggest*, taken in conjunction with temperature and acidity experiments, that the pathogenic importance of the worm is very slight, if not negligible.

del Rio's objection, that the consumption of infested vinegar is repugnant, can be admitted; a few simple precautions would probably restrict the worm to those places where it is valued and respected—namely to helminthological and biological laboratories. But in the opinion of the present writer there is no longer any need for alarm on the part of the general public.

III.—The Eelworm in the Vinegar Industry.

Vinegar was formerly made by storing weak wine or cider in large vats at a suitably high temperature in the presence of "mother of vinegar." The latter is a living ferment—a yeast—called *Mycoderma aceti*, and it appears, with various other bacteria and fungi, as a skin floating on the surface of the vinegar. It effects the necessary oxidation of alcohol to acetic acid—a process technically termed "acetification." The above is the Slow or Orleans process of vinegar manufacture, and although it still obtains in wine-growing districts it has been largely superseded elsewhere by the German or Rapid process. The essential unit of the latter is the "acetifier"—a large vat loosely filled with beech-wood twigs and chips which form a matrix on which the mother grows; a suitable alcoholic fluid is continuously sprayed over this matrix from a rotating distributor at the top of the vat and is slowly oxidized by the mother as it trickles through the twigs. The fluid is collected at the bottom of the acetifier and returned to the distributor again: it is circulated through the acetifier repeatedly until the required percentage of acetic acid is produced—a process occupying some two or three weeks. The loose matrix provides a very large area of contact between the mother and the alcoholic fluid, and also conduces to thorough aeration—and therefore to rapid oxidation. The heat induced by this latter chemical reaction tends to accelerate the reaction within limits, and, as the acetifier is well ventilated top and bottom, it gives rise to an efficient circulation of air by convection—thus ensuring a copious supply of the necessary oxygen. It will be readily understood, therefore, that this Rapid process has distinct advantages over the older Orleans process.

It was formerly thought that the eelworm occurred only in the vats of the Orleans process. Thus Davaine states that they live always in

vinegar made from fruit, "whence it happens that, previously very common, they are to-day very rare." As has been noted above, Lindner finds them almost exclusively in Rapid Process vinegar, while Pallecchi finds them chiefly in Orleans Process vinegar: the difference is evidently a geographical one for the most part. Henneberg finds the worms commonly in both processes, though rather more frequently in the Orleans process "where the more favourable nourishment, the lower acidity and the longer duration of vinegar-production conduce to particularly heavy multiplication. In such factories," Henneberg continues, "a thick white slime formed of innumerable eelworms can often be seen on the vat-walls above the liquid." The point concerning the "more favourable nourishment" to be obtained in the Orleans process is to be explained by the fact that, in Germany, raw spirit is often used as the source of alcohol (hence the term "Spritessig" for vinegar so produced), but even in this case a certain amount of vinegar or malt is added, for the mother is a living thing and requires suitable food.

Henneberg alone appears to have investigated the industrial importance of the worm with any thoroughness and his observations may be conveniently summarized here. In the Rapid process factories in Germany the worms live in the acetifiers ("Essigbildnern") on the walls and on the beechwood twigs, in places where they are not easily washed off by the vinegar flowing through; the temperature here is particularly favourable—usually about 20° (in Germany). If for any reason the flow of vinegar has been stopped for some time the number of worms increases enormously. When worms are added to an uninfested acetifier which is working normally, they fail to gain a footing and are washed out by the vinegar. The vinegar produced is usually diluted before being casked for sale, and after this dilution the number of worms rapidly increases. They are capable of destroying the delicate skin formed by the vinegar bacterium, and thus constitute a harmful influence in the process when present in large numbers.

Experiments show that the worms can be transferred from one vat to another by the agency of the vinegar fly, though since they resist desiccation they would scarcely be so conveyed over large distances, as from one factory to another. Henneberg traces this wider dissemination to the common practice of starting a new vat by inoculating it with

a culture of mother obtained from another factory: he therefore recommends the use of pure cultures of the vinegar bacterium for this purpose—a practice which he finds quite satisfactory.

The writer was fortunate in being able to inspect a large Rapid process factory in London where the methods employed were fully explained and demonstrated by the courtesy of the manager, who has also kindly given permission for the following details to be published.

At this factory the "raw material" is malted barley, that is, barley that has germinated and so has produced the enzyme "diastase." The initial processes result in the production of a light beer or "wort," and may be summarized as follows: diastatic fermentation converts the barley-starch into maltose, which hydrolyses to form glucose, and the latter is subjected to zymatic fermentation (by means of the ferments of brewers' yeast) by which it is converted into alcohol. The wort, containing about 6 per cent. alcohol, is matured for some time and then fed to the acetifiers. The latter (at the particular factory in question) develop a temperature of 40°–45° (*cf.* Henneberg's citation of 20° for the German factories), and it has been seen that such a temperature is fatal to the worm. Samples taken from the sprinkler of an acetifier at the factory showed no trace of it. Hence the question of the worm's destroying the film of mother does not arise in this case: the acetifiers are protected by their high temperature.

After storage (to acquire flavour) the crude vinegar is filtered through rape-seed or through beech chips in large vats: in one or two of the latter a small population of eelworms was found. Next, however, the vinegar is filtered under pressure through discs of highly compressed cotton pulp, and this process effectively removes the worms. The clean vinegar is now stored in vats until required for bottling or casking. Strangely enough, a few eelworms were found in a sample from one of these storage vats. Finally the vinegar is sterilized in a multiple-tube steam sterilizer at a temperature of 88° (which must be instantly fatal to the eelworm), and is then delivered to the casks and bottles: both of the latter are steam-sterilized before being filled. The writer finds that bottled vinegar from this factory is entirely free from the eelworm (as would be expected), yet they are occasionally to be found in vinegar from the casks.

The distribution of worms throughout the process may now be tabulated :—

Preparation of wort	No worms found.
Acetification at 40°-45°	No worms found.
Filtration (vats)	Worms found.
High-pressure filtration	Worms removed.
Storage (vats)	Worms reappear.
Steam sterilization at 88°	Worms destroyed.
Delivery into (*a*) Bottles	No worms found.
(*b*) Casks	Worms reappear.

In view of these facts it seems probable that the worm is introduced anew into the vinegar at the various stages of its manufacture.

Del Rio, it may be recalled, suggested that the presence of the eelworm in vinegar indicated that the vinegar was unduly weak in acetic acid (less than 4 per cent.), and that it was manufactured under conditions of doubtful cleanliness. The writer found that a sample of vinegar made at this London factory (and purchased at a shop) was of 5 per cent. strength ($\pm$ 0·1 per cent. by titration with phenolphthalein : total acidity as acetic acid), so that del Rio's criticism does not apply on this score. In the matter of cleanliness also, the factory appears to be above suspicion. Throughout the process the vinegar is conveyed from one part of the plant to another in pipes until finally it is run from taps into the casks or bottles : at nó stage is it handled or unnecessarily exposed. Most of the vats require to be ventilated, and it is highly probable that the worms are introduced through the various air-holes provided.

In the yard of the factory vinegar flies (*Drosophila* species) were to be seen in fairly large numbers. Three of these were captured and placed in an observation box in which were dishes of vinegar infested and uninfested by the vinegar eelworm. It was thought that the flies might convey the worm from one dish to the other ; but unfortunately they were all drowned in the vinegar within a few days without having accomplished this. Henneberg claims to have demonstrated this mode of transference, however, and it is at least possible that the responsibility for reintroducing the worm at the various stages of the process rests on the fly.

Unwin (1907) has investigated the life-history of the true vinegar fly, *Drosophila funebris*, and a few relevant facts may be quoted from his paper. The eggs hatch in about three days; the maggots are full grown in about three weeks; pupation occurs naturally in soil and occupies about a week in summer, but the pupæ may hibernate in the soil: hibernation may also occur in the winged state. The eggs are usually laid in rotting fruit. Imms (1925) states that, in the case of *D. ampelophila*, the eggs are frequently submerged in fluid, but Unwin could induce *D. funebris* to lay only in fruit that was not too moist. Unwin also states that *D. funebris* can carry bacteria, *Mycoderma aceti*, etc., from one fluid mass to another. The adult is a small brown fly, about 1/5 inch long, and with bright red eyes: it has a proboscis of the general muscid type. Dealing with the subject of chemotropism, Imms quotes Barrows (1907) to the effect that *D. ampelophila* displays a maximum response to a mixture of 20 per cent. alcohol and 5 per cent. acetic acid; he goes on to say: "It is noteworthy that cider vinegar and fermented cider contain alcohol and acetic acid in percentages very close to those just quoted."

In the yard of the vinegar factory was a heap of twigs and chips from one of the acetifiers. This might well provide a suitable breeding ground for the vinegar fly if it was left in the yard long enough—a matter of about a month in summer.

Henneberg implies that the eelworms are carried on the legs of the fly: he does not state whether he looked for them in the gut. It seems theoretically possible that the worm might be carried alive in this way, in which case it could be transported over considerable distances. The lateness of the season has prevented the writer from pursuing this line of enquiry any further at present; but in this connection the worm's habits of coming to the surface of vinegar and of burrowing into crevices are suggestive.

The anomaly of the worm's presence in the casks and of its absence in the bottles of vinegar is difficult to explain. The casks are sterilized by being inverted over a steam-nozzle for about three minutes: at the end of this time they are quite hot to the touch on the outside, so that it seems improbable that any worms inside the cask can withstand such rigorous treatment. More probably the worms are introduced anew into

the casks after sterilization, for the casks are stacked in the open, without bungs, for several minutes until they are required for filling. It would be interesting to know whether the worms could be excluded from the casks altogether simply by inserting the bung immediately after sterilization, and removing it only for the process of filling. If the worms were present in the delivery vats, they would appear as commonly in the bottles as in the casks, which is not the case.

Thus, in conclusion, the vinegar eelworm can be excluded from the acetifiers (where alone they can be proved definitely harmful to the manufacturing process) provided that a temperature of about 40° is maintained. Mitchell (1916) states that 160° F. (71° C.) is fatal to the worm, but this is clearly an over-estimation.

The worms appear to have been satisfactorily excluded from the bottled vinegar, and it is probable that a few additional precautions in the filling of the casks would remove them from here also. This should allay any apprehensions on the part of the consumer.

The complete eradication of the worm from all stages of the manufacturing process would be very difficult. It would involve not only a thorough disinfection of the entire plant, but also (probably) a campaign against the vinegar fly. On the whole, the end does not seem to justify the means. In the case of setting up a new factory it should be possible to prevent the worm from gaining a footing : the use of pure cultures of the vinegar bacterium, as Henneberg has suggested, and precautionary measures to exclude the vinegar fly (such as the protection of vats by means of wire gauze over ventilation-holes), should have the desired effect.

The question of the worm's original introduction into a medium apparently so unnatural as vinegar is not of practical importance, and may be relegated to a final section.

IV.—The Origin and Dissemination of the Eelworm.

The question of the worm's ultimate origin is chiefly a matter for surmise, but some of the theories expounded in the literature are not without interest. Dujardin noted that several species of *Rhabditis* (in which genus he included the vinegar eelworm) have a very exclusive habitat. " One cannot help supposing," he said, " that they must at

first arise spontaneously in the medium in which they afterwards continue their existence by reproduction." Davaine quoted Buffon to the same effect, but himself remained sceptical: he failed to produce eelworms in flasks of vinegar kept in contact with the air for ten years. From experimental cultures in sugar solutions and in fruits and vegetables he came to the conclusion that the worms arrive in vinegar continuously by way of the grapes used for making wine. He found they would live in moist soil and in such vegetable "roots" as the beet and onion, so he imagined that this was their natural habitat and that they passed into the grape when (for instance) a bunch happened to touch the ground. In support of this he asserted (wrongly, as has been shown) that the worms appear only in vinegar made from fruits. del Rio also suggested that kitchen-waste, or earth rich in humus, or possibly fruits, might be the natural medium of the worm. Henneberg stated that the worm had not been observed in nature and considered that it might be a species which had become exclusively adapted to living in vinegar.

Davaine's theory would be more acceptable if the vinegar eelworm had ever been recorded from fruit or from the soil, or from fresh wine or cider: the writer has been able to find no such record. Moreover, the theory would fail to explain the worm's presence in vinegar made from malt or from raw spirit. The important point in this connection is that there is no need for the worm to be continuously introduced into a factory. Once established there it could maintain itself indefinitely: possibly it has lived exclusively in vinegar ever since it first appeared there—a matter of several centuries. In this case it would be disseminated with samples of the mother, or by the vinegar fly. There would still remain the question of its ultimate origin.

In stating that the worm has not been observed in nature, Henneberg seems to have overlooked a definite reference to it on the part of Ludwig (1886). Ludwig was working on the so-called "slime-fluxes" of trees—slimy exudations appearing on the trunk and containing numerous bacteria, yeasts, fungi, protozoa, and so on. In the reference cited (p. 170) Ludwig says, referring to the slime-flux of the oak: "In dem Schleime finden sich dann sehr zahlreich Essigälchen." According to de Man (1910), Ludwig sent these worms to Leuckart, who replied by stating that he regarded them as a new species and would propose the name *Rhabditis dryophila*. In 1910 de Man demonstrated their close

similarity to the true vinegar eelworm, and made them a variety, *dryophila* of that species. In the same publication de Man described a new species, *ludwigii*, of the same genus and from the same white slime-flux of the oak: it appears to have closer affinities to the sour paste eel than to the vinegar eel.

These nematodes of the slime-fluxes seem to the writer to offer a possible solution to the problem of the vinegar eelworm's ultimate origin. From information and references courteously provided by Mr. J. Ramsbottom, O.B.E., the following relevant facts may be given concerning these slime-fluxes.

Red, brown and white fluxes are commonly distinguished. The first two are apparently always due to wounds or frost-cracks and consist of exudations from the actual wood, while the white fluxes are totally confined to the bark (arising in the phloem) and can apparently spring from uninjured bark. Ogilvie (1924) states that insect larvæ, protozoa and nematodes are common in the red and brown fluxes: the latter require a year for their full development. Sugars are absent from them or present only in traces. The red flux of the elm gives a hydrogen ion exponent of pH: 9·0–9·5. The white flux also contains a variety of organisms (nematodes not specified), but it lasts only a few months, usually from June to August; sugars are absent from the white flux of the willow (pH: 6·0–6·7), but that of the elm contains a considerable amount of glucose. A full account of the microflora inhabiting these fluxes is given by Ludwig (1886) and by Hansen (1889).

The white fluxes normally ferment, producing first alcohol and then acetic acid (compare the chemistry of vinegar manufacture, p. 27), and the strong smell of beer and vinegar attracts flies which probably spread the flux. The white fluxes occur on oaks, beeches, and many other trees (see Ogilvie).

Vinegar, then, is not such an unnatural medium as some have supposed. It is to be found in nature, and containing an eelworm which differs from the vinegar eelworm only in the proportions of the body and of the female genitalia (the uterus extends further forwards in the variety *dryophila*): the spicules and papillæ in the male are indistinguishable in the two varieties. A difficulty is presented in the short life of the white slime-flux: Ogilvie mentions nematodes in the red flux, which

lasts the year round, but these have apparently not been described. It is at least possible that the same eelworms occur in both fluxes, for the worms of the white flux must have some means of existence during the winter months. Such information as is available regarding the nature of the red fluxes (hydrogen ion Exponent, absence of sugar) at least suggests no factor which would prevent the closely related vinegar eelworm from living in them. And, finally, if the vinegar fly can convey vinegar eelworms from vat to vat, why should it not convey flux eelworms from tree to tree? Reliable information is required on these points: the species of eelworm inhabiting the red fluxes, the species of fly visiting the red and white fluxes, and the possibility of the same individual fly visiting both red and white fluxes and conveying eelworms from one to the other. The writer has been, and still is, on the look-out for slime-fluxes, but has not succeeded in finding any up to date.

Since the white flux occurs on beeches and since beech twigs are used in the acetifiers it might be supposed that the eelworms have been introduced into the factories in this way. Against such a theory must be set the following points: (*a*) Eelworms were found in vinegar long before the Rapid process (which alone employs beech twigs) was invented; (*b*) neither the variety *dryophila* nor the species *ludwigii* has, to the writer's knowledge, been recorded from vinegar; and (*c*) fluxes seem to arise on the main branches of trees rather than on the twigs.

Henneberg has vouched for the fact that the vinegar fly can carry eelworms from one vat to another. Conte (1900a) has shown how the size and proportions of a free-living nematode (*Rhabditis monhystera*) can be markedly changed by a change in environment, and Henneberg and de Man have recorded remarkable variations in the size of the vinegar eelworm. Taking all things into consideration, it does not seem extravagant to suppose that the vinegar eelworm was first introduced into vinegar centuries ago from its natural habitat in the slime-fluxes—possibly by the vinegar fly, and that the slight (almost certainly non-specific) variations have developed subsequently.

SUMMARY.

From considerations of previous work on the vinegar eelworm, and from the writer's experiments, it is concluded that:

I.—Concerning physical and chemical stimuli:

(*a*) The worm has very little need of oxygen, but displays a negative geotaxis which is independent of dissolved oxygen as a stimulus.

(*b*) It tolerates temperatures between 0° and 35°, can survive short intervals at 20°, and can live for several days at 37°. It is practically indifferent to light.

(*c*) The worm and its larvæ are very susceptible to drying, surviving only a few minutes a thorough desiccation from water.

(*d*) The toleration limits for hydrogen ion concentration are pH : 1·6 on the acid side and about pH : 11 on the alkaline side.

(*e*) So far as can be ascertained, the worm tends to make the medium in which it lives slightly but permanently more acid than it would otherwise be.

(*f*) It is very sensitive to iodine, ammonia and chloroform, but is extraordinarily resistant to most chemical substances, including acidity indicators and buffer solutions.

(*g*) It can be grown in most culture media, and has been cultured aseptically by Zimmermann, who finds that either bacteria (living or dead) or an unknown substance from the autolysate of yeast is essential as food.

II.—Concerning the worm's pathogenicity to man, evidence goes to show that it is practically harmless.

III.—Concerning the importance of the worm to the vinegar industry, it is demonstrably harmful only when it occurs in the acetifiers, from which it can be excluded by a suitably high temperature (40° C.). It can probably be entirely excluded from retailed vinegar without difficulty, but its complete removal from a factory in which it is established is likely to be onerous.

IV.—Concerning the origin of the eelworm in vinegar, nothing is known, but some suggestions are made.

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On the Morphology of *Heterodera schachtii* with Special Reference to the Potato-strain.

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Introduction.

Heterodera schachtii Schmidt, 1871, first discovered and studied by Schacht in 1859 was recognised as a parasite injurious to the sugar-beet by Kühn in 1881. Subsequent to the discovery of its pathogenicity it has been studied by many observers both in Europe and in the United States where it was introduced about the beginning of the present century, and has now become established in the beet-growing areas of Utah and California. A detailed morphological account of the nematode was first given by Strubell, 1881, who carried out his observations on material obtained from sugar-beet. Other workers have supplemented Strubell's description in several minor respects and have also described the nematode as it occurs on other plant species.

Since its first recognition as a plant parasite, *H. schachtii* has been reported as attacking, in addition to sugar-beet, a great variety of plants, including cereal and other agricultural crops. Amongst these occur the following, oats and wheat (Kühn 1874), barley (Hollrung 1890), lucerne (Hollrung 1898), hops (Percival 1895), rye, many species of Brassica, and potato (Kühn 1881). The degree of susceptibility of these and other plants to the attacks of the nematode, has been found by various observers to differ widely. Thus for example, a list of non-susceptible plants drawn up by Vanha and Stoklasa (1896) includes twenty-three species, including the potato, which appear in a list of susceptible species given by Marcinowski (1909).

Investigations have proved that this is due to the existence of biological or physiological strains such as have been shown by Steiner (1925) to

occur also in *H. radicicola* and *Tylenchus dipsaci*. These strains are, however, particularly well marked in *H. schachtii* and Wollenweber (1923), after working with a strain obtained from Mecklenburg, which is very highly specialised on the potato, has established a new species *H. rostochiensis* ad int. which he bases not only on physiological specialisation of the strain but also on morphological characters.

A strain of *H. schachtii* occurring on potatoes in the Lincolnshire area has been under observation by members of this Institute since 1924, and appears to have reached an equal degree of specialisation, since, up to the present, all attempts to produce an infection in plants other than the potato have proved negative.

A detailed morphological study of this Lincolnshire strain has been carried out with a view to establishing whether adequate grounds exist for the retention of Wollenweber's species *H. rostochiensis*. The results of these observations have been compared with the findings of other workers, and, to facilitate the comparison, material obtained from sugar beet and oats has been concurrently studied. Unfortunately only preserved material of these latter strains was available and certain stages of the life-cycle were not obtained.

Morphology.

The brown cyst.

Cysts were isolated from Lincolnshire soil and also from the plot experimentally infected with the Lincolnshire strain at the Institute's Field Station in Hertfordshire. Morgan's flotation method was used for extracting the cysts from the soil. Although a great number of cysts were measured it was found impossible, in view of the great variation in size, to establish definitely the maximum and minimum dimensions of this strain.

Cysts isolated from Lincolnshire soil varied in size from 0·955 mm. by 0·8 mm. to 0·132 mm. by 0·109 mm., the average size being 0·576 mm. by 0·405 mm. The ratio of length to breadth varied from 1 : 0·85 to 1 : 0·57, the average ratio being 1 : 0·692, *i.e.* roughly the average cyst was three-fifths as broad as long.

From the experimental plot in Hertfordshire the maximum and average sizes of the cysts were found to be considerably smaller. This was

possibly due to differences in soil composition and cultural methods. They varied from 0·477 mm. by 0·382 mm., to 0·05 mm. by 0·036 mm., the average size being 0·317 mm. by 0·245 mm., and the average ratio of length to breadth was 1 : 0·77 or, the breadth roughly four-fifths of the length.

Unfortunately only white cysts were present in the preserved material from beet and oats so a direct comparison could not be made. The following table, however, shows the dimensions recorded by other observers for these strains.

Measurements recorded for the brown cysts of *H. schachtii* in mm.

Author.	Host plant.	Minimum length.	Maximum length.	Breadth.	Average.
Voigt 1892, 1894	Beet ...	0·6	1·0		
Marcinowski 1909	Beet ...	0·39	0·88		
Strubell 1888 ...	Beet ...	0·8	1·3	0·5–0·9	
Chatin 1891 ...	Beet ...	0·8	1·3	0·5–0·9	
Voigt 1892, 1894	Oats ...	0·5	0·8		
Marcinowski 1909	Oats ...	0·4	0·78		
Wollenweber 1923	Potato...				0·835 × 0·543
Lincolnshire strain	Potato...	0·05	0·95	0·03–0·8	0·447 × 0·325

In addition to the figures given above it is interesting to note that Liebscher (1890, 1892) states that he found a differentiation in the size of nematodes occurring on beet and oats from those found on peas and vetch, the former producing larger eggs, larger larvæ, and larger, shorter-necked females. He gives, however, no measurements. Marcinowski believed that greater differences would appear in the morphology of the strain if they were permitted to continue for a greater number of generations on the same host plant.

Marcinowski (1909) also gives for the beet and oat strains studied by her, figures relating to the ratios of the various parts of the brown cyst which demonstrate the general body form. Similar measurements

have been carried out for the potato-strain and the results are shown in the following table.

Ratios of dimensions of the brown cysts of *Heterodera schachtii*.

	Length / Thickness.			Length of Neck, in mm.			Length / Length of Neck.		
	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
Beet Marcinowski	1·26	1·97	1·55	0·05	0·16	0·09	4·6	10·8	7·0
Oats Marcinowski	1·57	2·20	1·93	0·09	0·16	0·12	3·3	7·7	5·38
Potato	1·06	1·76	1·37	0·014	0·018	0·064	0·018	0·053	0·034

As will be seen from the above tables, the females or brown cysts of *H. schachtii* from the beet are rather longer and relatively thicker than those from the oat, with the neck both actually and relatively shorter than in the latter form. The Lincolnshire potato-strain, which has a much greater variation in size, may almost attain the maximum size of the beet-strain, but is, at its minimum, only one-third of the minimum size recorded for the oat-strain, and, further, it is relatively much broader and shorter-necked than either. Wollenweber gives only the average dimensions of the potato-strain from Mecklenburg and these are almost double the writer's findings for the Lincolnshire-strain. It is, however, worthy of note that the potato-strains agree in general body form, both tending to produce almost spherical cysts.

The Eggs.

As stated above, Liebscher (loc. cit.) found that nematodes from oats and beets produced rather larger eggs than nematodes from peas and vetch. Strubell and Chatin found that the eggs of the beet-strain nematodes measured 0·08 mm. by 0·04 mm. Wollenweber gives the size of the eggs of *H. rostochiensis* as 0·097 mm. by 0·040 mm., and remarks that the eggs of *H. schachtii* from the beet are somewhat larger, from rye somewhat smaller.

In view of this conflicting evidence it was decided to test the range of variation in the size of the eggs of *H. schachtii* of the Lincolnshire-strain. Brown cysts were isolated and crushed between slide and coverslip to liberate the embryonated eggs. Fifteen of these, selected

at random from each cyst, were then measured. By this means it was found that although considerable dimensional variations existed, the eggs produced by each female were of approximately equal size, and furthermore, that the size of the eggs bore no relation to the size of the containing cyst. These facts are illustrated by the following examples.

Cyst A.—Size, 0·34 mm. by 0·25 mm. ; eggs, 0·104 mm. by 0·045 mm. to 0·096 mm. by 0·045. Average size, 0·099 mm. by 0·046 mm.

Cyst B.—Size, 0·16 mm. by 0·15 mm. ; eggs, 0·114 mm. by 0·050 mm. to 0·100 mm. by 0·045 mm. Average size, 0·096 mm. by 0·049 mm.

Cyst C.—Size, 0·27 mm. by 0·21 mm. ; eggs, 0·073 mm. by 0·032 mm. to 0·068 mm. by 0·027 mm. Average size, 0·069 mm. by 0·028 mm.

From the complete series of these measurements the eggs were found to vary from 0·0l4 mm. to 0·068 mm. in length and from 0·05 mm. to 0·027 mm. in breadth.

Similar results were obtained from observations carried out on the preserved beet and oat material. Eggs from the beet-strain varied from 0·123 mm. to 0·73 mm. in length by 0·050 mm. to 0·027 mm. in breadth, *i.e.*, they slightly exceeded, both in minimum and maximum dimensions, the size of the eggs obtained from the potato-strain, thus agreeing with Wollenweber while differing from Strubell's observations. Eggs from the oat-strain were found to be smaller than in either of the foregoing forms and also showed a lesser degree of variation, viz., from 0·091 mm. to 0·073 mm. in length, by 0·032 mm. to 0·025 mm. in breadth.

Zimmermann (1927, 1928) records a nematode strain which, having been highly specialised on potato, was becoming readapted to beet, producing eggs of a peculiar curved form which was not observed in the eggs of the strain infecting the original host. The three strains studied by the present author showed no differences in the shape of the eggs which were in every case slightly concave on one side.

The First Stage Larva.

Conflicting evidence is also abundant in the literature concerning the morphology of the first-stage larva. Thus, although Strubell and Chatin give the size of the newly-hatched larva as 0·36 mm. by 0·16 mm. and 0·35 mm. by 0·15 mm. respectively, Wollenweber states that the larva of *H. rostochiensis* measures 0·372 mm. by 0·018 mm. and is *smaller* than the well-known *H. schachtii* forms. On the other hand

Wollenweber's statement that the mouth-stylet of the larva, measuring from 0·016 mm. to 0·019 mm. in length, is comparatively shorter than that of the beet-strain, agrees with the findings of Strubell and Chatin who give the length of the stylet from beet as 0·023 mm. and 0·022 mm. respectively.

The newly hatched larvæ of the Lincolnshire potato-strain were found to vary from 0·268 mm. to 0·490 mm. in length and from 0·013 mm. to 0·027 mm. in width. Only about five per cent. were less than 0·409 mm. in length by 0·018 mm. in width, the average size being 0·458 mm. by 0·026 mm. The stylet showed a range in length of from 0·011 mm. to 0·027 mm., being, in the majority, not less than 0·021 mm. long and not strictly proportional with the length of the larva. The larvæ of the Lincolnshire potato-strain, therefore, exceeded Wollenweber's strain by up to 0·128 mm. in length, and were correspondingly thicker and furnished with larger mouth stylets.

Embryonated eggs from both beet and oat material were obtained and crushed between slide and coverslip to liberate the larvæ. These were then measured for purposes of comparison. Larvæ thus obtained from the beet had an average length of 0·59 mm., the maximum length 0·318 mm. being slightly less than the size given by Strubell. The average length of the stylet was 0·025 mm. Larvæ from the oat material averaged 0·289 mm. with a maximum length of 0·317 mm., the average length of the stylet being 0·027 mm.

With regard to general morphological characters the larvæ of the potato-strain under observation were found to correspond in all particulars with those obtained from beet.

The Adult Male.

Wollenweber gives no description or measurements of the adult male of *H. rostochiensis*, but bases the species on morphological grounds solely on the characters of the cyst, egg and larva. Previous workers have, however, given detailed anatomical descriptions with measurements of the sugar-beet-strain.

Strubell gives the length of the adult male as 0·8 mm. to 0·9 mm. occasionally up to 1·0 mm. The stylet is given as 0·03 mm., and the spicules as 0·033 mm. in length but no measurement is given for the gubernaculum. Chatin gives 0·8 mm. as the average length, 0·029 mm. as the average length of the stylet and 0·033 mm. average length of

the spicules. Marcinowski found that although the adult males were commonly between 0·8 mm. and 0·9 mm. long they occasionally attained a length of 1·37 mm. In studying preserved material from beet, the present author found males to vary from 0·727 mm. to 0·777 mm. in length, with stylets of from 0·013 mm. to 0·035 mm., spicules from 0·036 mm. to 0·04 mm., and gubernaculum from 0·008 mm. to 0·011 mm. in length. The ratio of breadth to length was 1 : 34 as compared with 1 : 48 of Marcinowski and 1 : 32 of Strubell. Males from oat material were slightly larger, reaching a maximum of 0·818 mm. with the stylet up to 0·031 mm. and spicules of from 0·033 mm. to 0·038 mm.

Adult males obtained from the Lincolnshire potato-strain were found to be smaller than in any case previously mentioned. They ranged from 0·591 mm. to 0·704 mm., and had an average length of only 0·641 mm. The average ratio of breadth to length was 1 : 31. The stylet varied up to 0·035 mm., spicules from 0·037 mm. to 0·042 mm. and the gubernaculum from 0·010 mm. to 0·015 mm. in length.

A summary of the dimensions of the adult male of *H. schachtii* according to Strubell, Marcinowski, and the findings of the present author is given in the table below.

Dimensions of adult male of *Heterodera schachtii* in mm.

Author.	Host.	Max. Length.	Average Length.	Length. / Breadth.	Stylet.	Spicule.	Gubernac.
Strubell ...	Beet...	1·0	0·8–0·9	32	0·03	0·033	
Chatin ...	Beet...		0·8		0·029	0·033	
Marcinowski	Beet...	1·37	0·8–0·9	48			
Triffitt ...	Beet...	0·777	0·728	34	0·035	0·04	0·011
Triffitt ...	Oats...	0·818	0·786	33	0·031	0·038	0·009
Triffitt ...	Potato	0·704	0·641	31	0·035	0·042	0·015

As in the case of the larva, the general morphological characters were found to be identical in all major particulars for the three strains. The labial region and the stylet and buccal musculature were particularly studied, as were also the spicules and gubernaculum. The cuticular striations in the labial and tail regions were difficult to distinguish in

the beet-strain, distinct in the potato-strain, and especially well marked in the oat-strain, but these differences were possibly due in some part to the reagents used in preservation of the beet and oat material.

The spicules were morphologically identical in the three strains. They were slender and sickle-shaped, with small rounded proximal extremities, and a double series of longitudinal ridges extending somewhat obliquely from the shoulder region to the distal end. In the case of each strain a simple gubernaculum was also present, rather closely applied to the spicules in the anal region. This was generally only visible in lateral view and was evidently overlooked by Strubell. The size and position of the various parts of the alimentary canal and the length and extent of the gonad varied very slightly in different individuals but was approximately identical for the three strains. The only marked morphological discrepancy was found in the development of the lateral wing-like outgrowths in the posterior region of the body. These were present in all strains, arising anterior to the proximal extremities of the spicules and extending downwards and outwards, forming thin lateral flanges to the tail. They were, however, in the potato-strain, consistently very much more strongly developed than in either of the other strains.

Cultural Experiments.

Repeated attempts have been made to produce an infection of sugar-beet with the Lincolnshire-strain by germinating beet seedlings in heavily

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Morphological Characters of the Adult Male of *Heterodera schachtii*.

Fig. 1.—Beet-strain nematode—Head.

Fig. 2.—Beet-strain nematode—Tail, ventral view, showing spicules and gubernaculum.

Fig. 3.—Beet-strain nematode—Tail, lateral view, showing spicules and gubernaculum.

Fig. 4.—Oat-strain nematode—Head.

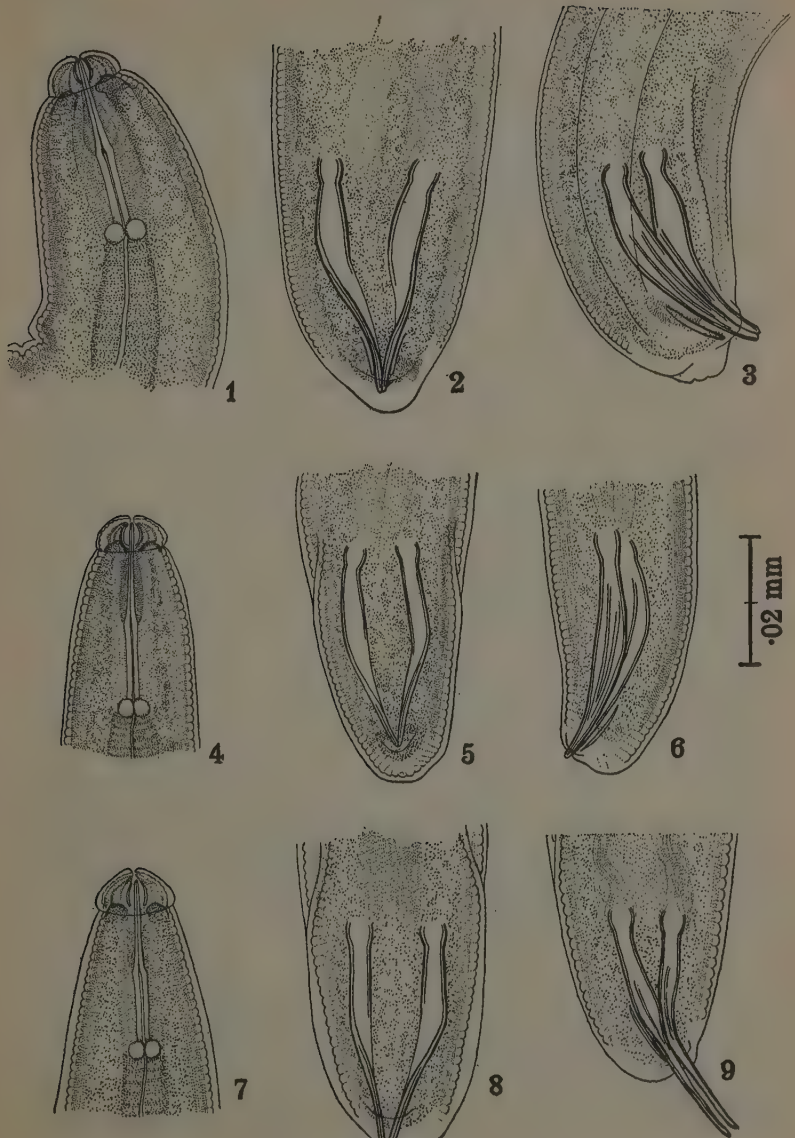
Fig. 5.—Oat-strain nematode—Tail, ventral view, showing spicules, cuticular striations and lateral flanges.

Fig. 6.—Oat-strain nematode—Tail, lateral view, showing spicules and gubernaculum.

Fig. 7.—Potato-strain nematode—Head.

Fig. 8.—Potato-strain nematode—Tail, ventral view, showing spicules and lateral flanges.

Fig. 9.—Potato-strain nematode—Tail, lateral view, showing spicules extruded, and gubernaculum.



Heterodera schachtii.

infected soil, but these have consistently given negative results. Successive crops of beet seedlings have been raised in the same infected soil over a period of eighteen months with a view to giving the nematode time to adapt itself to the new host, but, up to the present date, no infection has been discovered. Zimmermann (1927) found that, on cultivating sugar-beet for two successive years on land infected with a strain highly specialised on potato, although the first season's crop was not attacked, a heavy infection occurred during the second year. Contrary to the early completion of the nematode life-cycle on the potato, cyst-formation on the beet took place only late in the season. Further, although there was some proliferation of the lateral root-system, the beets did not show the distortion typical of nematode attack.

A third crop of beet grown on this plot in the following year was more heavily infected, a few of the plants showing distortion, although the majority of the crop retained the normal structure. The late completion of the life-cycle was again observed.

Zimmermann interprets the lateness of the life-cycle and the fact that the first crop was not attacked as an indication of the slow readaptation of the strain to the beet as a host-plant. He therefore concludes that *H. rostochiensis* is only a biological strain of *H. schachtii*, and not a true distinct species. He proposes, therefore, that the potato-strain should be named *H. schachtii*, var. *solani*.

This naming of the biological strains seems to the writer an unnecessary measure and one which would be likely to lead to confusion, for, as shown by Zimmermann himself, the potato-strain is transmissible to beet. It therefore seems reasonable to suppose that it is also transmissible to other plant species, and that, by becoming readapted over a great number of generations on the same host it might achieve an equal degree of specialisation upon the new host. Conversely, it seems equally possible that, with repeated changes of crop, a highly specialised strain might become comparatively omnivorous.

Discussion.

In summing up the differences between the various strains or races of *H. schachtii* from the data given above, it must be noted first, that though the dimensions of the brown cysts of the Lincolnshire potato-strain differ somewhat widely from those given by Wollenweber, the general body shape was found to be the same and to differ from the body

shape of the beet- and oat-strains as described in the literature. The potato-strain in both cases produces short, almost spherical cysts, while in the beet- and oat-strains the cysts are more elongated and more or less lemon-shaped.

The eggs are shown to exhibit such wide variations in size within each strain that any slight differentiations which may exist can hardly be regarded as specific in character. Similarly with regard to the first stage larva, size is proved to be of no material account, and, on general morphological characters the larvæ of the potato-strain remain indistinguishable from the larvæ of the beet-strain.

The characters of the adult male remain the same for all three strains with the possible exception of the size of the wing-like flanges of the tail and the intensity of the cuticular striations at the extremities. Since size is shown to be so very variable a factor within the species, any morphological distinctions which are made must therefore be based upon the anatomical structure of the worm. The similarity of spicular structure in the three strains hence assumes a greater significance than has apparently been accorded it by earlier workers.

Conclusions.

The shape of the brown cysts and the size of the caudal flanges of the male, therefore, remain as the only morphological differences between the strain of *H. schachtii* attacking the potato and the strains attacking beet and oats.

The shape of the brown cyst may yet be discovered to be dependent upon soil conditions or other external factors. That such factors do exist, and exert a modifying influence upon the morphology of the cysts, is proved by the alteration in size which has taken place on the transfer of the Lincolnshire potato-strain to Hertfordshire soil. In this instance the change of environment was made in the spring of 1926, when a quantity of heavily infected soil from Lincolnshire was laid down on the Hertfordshire plot. The subsequent cultivation of potatoes on this land has caused the infection to spread for a distance of about two yards, and in the autumn of 1927, *i.e.*, almost two years since the change of environment, the cysts show already a marked decrease in size.

The lack of morphological data, together with the physiological adaptability demonstrated by Zimmermann, seems to the author to prove conclusively that *Heterodera rostochiensis* Wollenweber, 1923 is not a true species and should therefore be regarded as a synonym for *Heterodera schachtii*.

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Journal of Helminthology, Vol. vi, *No.* 1, *March*, 1928, *pp.* 51-55.

A new species of *Phyllobothrium* van Ben., from an Alaska Dog Salmon with a note on the occurrence of *Crossobothrium angustum* Linton, in the Thresher Shark.

By Wm. P. Canavan.

(*Zoology Laboratory, University of Pennsylvania.*)

In connection with investigations of the United States Bureau of Fisheries in Alaska in 1906, Professor H. B. Ward spent considerable time in the study of the parasites of the Pacific salmon. Around Excursion Inlet he secured a large amount of valuable material. The following study was made of one of the specimens collected by him, to whom I am grateful for the loan of it.

A synopsis of the new species, *Phyllobothrium ketæ*, is given here :—

No hooks ; length of 97 cm. ; with four mobile, unarmed, peduncled bothridia attached by a broad base at the circumference of the thickened end of the scolex, their anterior margin being pinched in ; their free margins are curled and folded ; each has a round sessile sucker (acetabulum) anteriorly placed, almost half the size of each bothridium. It has a fifth vestigial sucker terminal ; a distinct neck present ; segments distinct ; the latter being 5 to 7 times broader than long. Genital pore is in the middle of the lateral margin ; ventral uterine pore present and central ; testes number from 110 to 135, in the medullary zone only. The cirrus is not spined ; normal type cirrus sac present. The vitellaria are numerous ovoidal structures extending nearly across the entire dorsal and ventral cortical layers of each proglottid, grouped somewhat laterally. The shell gland is dorsal to the ovary, which is bilobed ; the uterus lies with its long axis lateral. The eggs are ellipsoidal and average 40μ ; they have a filament, but no operculum.

The description of the genus as emended by Southwell (1925) is changed in such matters only that will aid the investigator to arrive at a more accurate conclusion, with the exception of his limiting factor : " Parasitic in Elasmobranch fishes, reptiles and mammals." The writer thinks it should read : " Parasitic in fishes, reptiles and mammals." None of the known species which have been referred to the genus, and of which Southwell gives a list, can be accepted as a species name for this. The latter, after studying my slides at my request writes : " I have seen that the species is new, but it bears a strong resemblance to *P. thridax* (*unilaterale*). It is also very closely related to *Calyptrobothrium*."

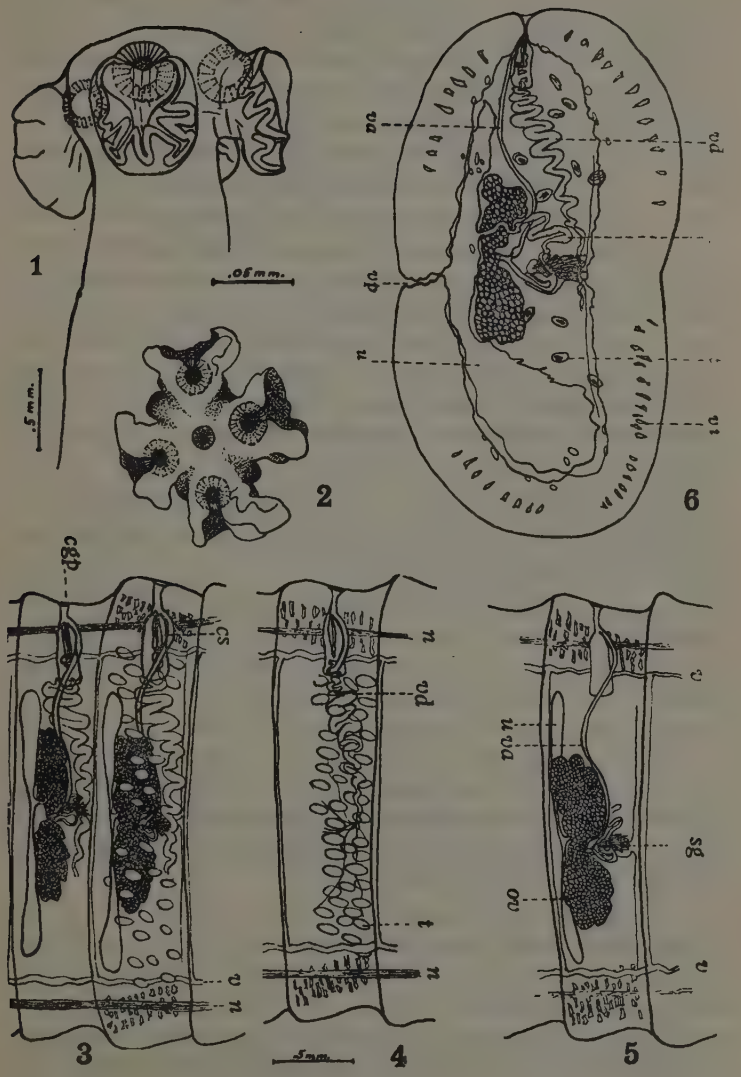
Segmentation of the strobila is distinct and regular, without an overlapping posterior border. In length, the strobila measures 97 cm., and in width, the gravid segments measure 3·78 mm.

The head is 1·43 mm. wide, and the neck 0·73 mm., with a length of 3·8 mm. No hooks are present. On the little protuberance, at the apex of the scolex, is a fifth vestigial sucker, noticeable only after manipulation of the head into position. The four bothridia, symmetrically arranged at the circumference of the thickened end of the scolex, have their accessory suckers in the top centre. These suckers are cup-shaped and sessile to the broad zone of the head. They have a diameter of 0·43 mm., which is about half the size of the bothridia.

Testes number from 110 to 135. They are semi-elliptical and vary in size from 40 to 60μ. The vas deferens arising anteriorly and a little beyond the median line, swells to about five times the size of the vagina and folds many times on its path laterally across the field. Before thecirrus sac it measures 50μ.

Phyllobothrium ketæ, sp. nov.

Fig. 1.—Scolex. Fig. 2.—Scolex, top view, showing fifth vestigial sucker. Fig. 3.—Mature segments ; reconstruction from c.l. drawings. Cs = cirrus sac, cgp = common genital pore, v = excretory vessel, n = nerve. Ventral view. Fig. 4.—Reconstruction from c.l. drawings of male structures. Vd = vas deferens, t = testes. Ventral view. Fig. 5.—Reconstruction from c.l. drawings of female structures. Sg = shell gland, ov = ovary, va = vagina, u = uterus. Ventral view. Fig. 6.—Reconstruction from 40 c.l. drawings of mature segment. Vi = vitellaria, uc = uterine canal, vp = ventral pore. Transverse.



Phyllobothrium ketæ, sp. nov.

A cylindrical organ constitutes the cirrus, which is doubled up to accommodate itself to the small space in the cirrus sac. The latter lies central and unilateral in the proglottid and is semi-rectangular in shape. The vagina arising from the common genital pore describes a curve anteriorly and mediad around the cirrus sac running in the dextro-sinister axis to the inter-ovarian space where it joins to the oviduct. It is dilated at a point proximal to the fertilization space where a simple slight bulging of its wall forms a receptaculum seminis.

The bilobed ovary lies in the mid-ventral area of the mature proglottid. From the median continuous connecting arch between the lobes the oviduct passes dorsally. At one-third of its course it receives the vagina. Just before the oötype it receives the common vitelline duct, the oötype being a continuation and slight thickening of the cubical cells of the oviduct, from which the lobate acini stand out. These acini form the shell-gland on the dorsal side only of the former. It is 0·29 mm. × 0·02 mm.

Vitellaria are embedded in the corticle layer ventro-laterally and dorso-laterally. They are in this case simple ovoidal structures that measure 60μ in mature segments.

The uterus, in the mature proglottid, is a long bilobed sac lying ventrally. In the gravid proglottid it is swollen to many times the size it attains there. The ventral pore is very evident in the latter. A number of eggs show various stages of development within the sectioned uterus. In total mounts they appear only as an opaque mass darkening the interior. They are ellipsoidal, without an operculum, but with a filament and three membranes, having an outer thin transparent membrane averaging 40μ in length, a second membrane, thicker than the first, retaining a fairly constant measurement of 30μ, and a third membrane around the segmenting ovum.

A consideration of the cortical layer and musculature of a mature segment shows several things of interest. The outside cuticle itself is a fibrous-like and fairly elastic structure, being translucent and a trifle jagged. The cuticular muscles are evident, inside of which lie the outer and inner fascicular layers of longitudinal muscles. An interlaced network of parenchyma and excretory tubules fills in the spaces around the above-mentioned cells and muscular bundles.

Coming into the widest part of the scolex, the layers of longitudinal muscles send out dorso-ventral fibres into the walls of the bothridia.

The nervous system consists of two strands parallel to the main excretory ducts, arising from two lateral ganglia in the scolex. Anteriorly, the two excretory vessels pass the ganglia by a zigzag course to terminate in the head from whence branches make a complete circuit, in a ramifying path, over and around each bothridium.

Host.—*Oncorhynchus keta* (Walbaun).
Location.—Pyloric cæca.
Locality.—Excursion Inlet, Alaska.
Type.—In collection of H.B. Ward, Urbana, marked Ex 24 b from Male Dog Salmon.

Not often does one get the opportunity to search through a 14-foot Thresher Shark of the species *Alopes vulpes* (Gmelin) for parasites. It was my pleasure to do so this Summer at Woods Hole with Dr. Edwin Linton at the Bureau of Fisheries there. In spite of the enormous size of the host, no entozoa were found in the cavities, except in the spiral valve, which yielded fifty-two tapeworms of one species. They averaged 18 mm. in length, and proved to be *Crossobothrium angustum* Linton. Professor Linton remarked during this recovery process, "Torpedos and Sand Sharks always have numerous parasites whereas Thresher Sharks rarely have any."

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Journal of Helminthology, Vol. vi, *No.* 1, *March*, 1928, *p.* 56.

Note on the Occurrence of *Ophiotænia lönnbergii* in Pennsylvania.

By Wm. P. Canavan.

(*Zoology Laboratory, University of Pennsylvania*).

Last Fall my attention was called to an adult *Necturus maculosus* Raf. in our vivarium by Dr. C. L. Parmenter, in charge, from the cloaca of which protruded some mature tapeworm segments. One cestode only was found in the duodenum which measured 83 mm. The head was easily recovered. It proved to be *Ophiotænia lönnbergii* (Fuhrmann) as described and illustrated by La Rue (1914). La Rue has frequently found the species in Necturus from Ohio and Indiana. This one is from Allegheny in Pennsylvania, showing a wider distribution. La Rue states: "I have examined your slides of *Ophiotænia* from *Necturus* and find them to be *O.lönnbergii* without question."

Reference.

La Rue, G. R., 1914.—"A Revision of the Cestode Family Proteocephalidæ." *Illinois Biol. Mono.* I, *p.* 214.

Journal of Helminthology, Vol. vi, *No.* 1, *March*, 1928, *pp.* 57-58.

Amendments to the International Rules of Zoological Nomenclature.

IMPORTANT NOTICE TO ZOOLOGISTS, PHYSICIANS, VETERINARIANS, AND OTHERS USING ZOOLOGICAL NAMES.

(*From the Public Health Reports, October 28th, 1927, pp. 2639-2640, issued by the United States Public Health Service.*)

Upon unanimous recommendation by the International Commission on Zoological Nomenclature, the International Zoological Congress which met at Budapest, Hungary, September 4th to 9th, 1927, adopted a very important amendment to article 25 (Law of Priority) which makes this article, as amended, read as follows (*italicized type represents the amendment;* roman type represents the old wording) :—

Article 25. The valid name of a genus or species can be only that name under which it was first designated on the condition—

(*a*) That (*prior to January 1st, 1931*) this name was published and accompanied by an indication, or a definition, or a description; and

(*b*) That the author has applied the principles of binary nomenclature.

(*c*) *But no generic name nor specific name published after December 31st, 1930, shall have any status of availability* (*hence, also, of validity*) *under the rules, unless and until it is published either—*

(1) *With a summary of characters* (*seu diagnosis; seu definition; seu condensed description*) *which differentiate or distinguish the genus or the species from other genera or species;*

(2) *Or with a definite bibliographic reference to such summary of characters* (*seu diagnosis; seu definition; seu condensed description*). *And further—*

(3) *In the case of a generic name, with the definite unambiguous designation of the type species* (*seu genotype; seu autogenotype; seu orthotype*).

The purpose of this amendment is to inhibit two of the most important factors which heretofore have produced confusion in scientific names. The date January 1st, 1931, was selected (instead of making the amendment immediately effective) in order to give authors ample opportunity to accommodate themselves to the new rule.

The Commission unanimously adopted the following resolution :—

(*a*) It is requested than an author who publishes a name as new shall definitely state that it is new, that this be stated in only one (*i.e.*, in the first) publication, and that the date of publication be not added to the name in its first publication.

(*b*) It is requested that an author who *quotes* a generic name, or a specific name, or a subspecific name shall add at least once the author and year of publication of the quoted name or a full bibliographic reference.

The foregoing resolution was adopted in order to inhibit the confusion which has frequently resulted from the fact that authors have occasionally published a given name as " new " in two to five or more different articles of different dates—up to five years in exceptional cases.

The three propositions submitted by Dr. Franz Poche, of Vienna, failed to receive the necessary number of votes in commission to permit of their being recommended to the Congress. Out of a possible eighteen votes for each proposition, Poche's proposition I. received nine votes, II. received six votes, and III. received seven votes.

Zoological, medical, and veterinary journals throughout the world are requested to give to the foregoing the widest possible publicity in order to avoid confusion and misunderstanding.

C. W. Stiles,
Secretary to Commission.

Journal of Helminthology, Vol. VI, *No.* 2, *June*, 1928, *pp.* 59-76.

On the Control of the Root Knot Eelworm, *Heterodera radicicola* Müll.

By Herbert W. Miles, M.Sc (Bristol), N.D.A.

and

W. H. Turner, B.Sc. (Sheffield).

Introduction.

The Root Knot Eelworm, *Heterodera radicicola* Müll., is widely distributed throughout the world, Marcinowski (8) quoting records of its occurrence in Europe, North America, Africa, Australia, Brazil, Chili, Madagascar, Sumatra, and Java. In warm regions the eelworm occurs out of doors infesting a variety of plants (6 and 8), and in the colder regions of Europe and North America the pest is prevalent under glass. In the British Isles the Root Knot Eelworm is common in the soil of commercial glasshouses devoted to the production of tomatoes and cucumbers. Though these two crops appear to be the only ones to suffer serious infestation in this country beans may also be attacked when grown under glass in infested soil; this commonly occurs in the Worthing area in Sussex and Hodson (7) records such attacks in Devon and Cornwall. Certain weeds growing in glasshouses may also be infested, notably docks, *Rumex* spp., bindweeds, *Convolvulus* spp., and knot grass, *Polygonum persicaria*.

Under the stimulation of the parasite, infested roots become swollen and galled, the galls varying in size from a pin's head to a walnut; these galls often coalesce and result in distorted, misshapen root systems with the fibrous roots reduced to a minimum. In severe cases the growth of the roots is interfered with and their functions interrupted, the plant growth is retarded, and wilting, yellowing of the foliage and death may result. The normal progress of the eelworm disease is, however, complicated and aggravated by the invasion of fungi such as *Colletotrichum tabificum*, which accelerate wilting, yellowing and death, often with the total loss of crop on infested plants.

Examination of tomato crops in various parts of the country indicates that under optimum conditions for plant growth tomatoes may be comparatively heavily infested with *H. radicicola* without the yield being appreciably diminished; the plants may, however, exhibit slight dwarfing, the foliage appear greyish green, and in warm weather wilt more readily than uninfested plants: symptoms suggesting deficient root action. Under less favourable conditions for plant growth the result of Root Knot Eelworm infestation is more obvious, and when attack by fungi occurs at the same time as eelworm infestation considerable loss in yield and in plants results. The association between plant infesting eelworms of the genus *Heterodera* and certain fungi: in the case of potatoes *H. schachtii* var. *rostochiensis* and *Rhizoctonia solani* (11 and 12), and in tomatoes *H. radicicola* with *Colletotrichum tabificum*, is becoming more pronounced and more widely recognised (17). Thus eelworms, though possibly only mildly injurious in themselves, as instanced by *H. schachtii* on hops (4), become of considerable importance when viewed as factors predisposing the plants to the attacks of fungi.

Experiments by Russell and Petherbridge (14 and 15) showed that *H. radicicola* and other species of eelworms could be killed by heating infested soil to a temperature of 140°F. Partial soil sterilization by heating is possible under commercial conditions but the operation is costly and the practice is followed only periodically even on the most progressive nurseries; therefore there appears to be a need for a simpler and cheaper means of control which could be carried out with greater frequency than the method of steam sterilization. Robson (13) working at the East Anglian Institute of Agriculture at Chelmsford,

found that relief was obtained by using sodium cyanide, therefore when calcium cyanide in granular form suitable for soil fumigation became available in this country it was thought that this substance might be worth testing as a vermicide for use under glasshouse conditions, especially as other experiments (11) had indicated that it might prove of value.

In 1925 the experiments herein described were commenced. These experiments have been essentially practical, simply designed and carried out under commercial conditions with the help and co-operation of interested growers. The authors desire to express their thanks to the growers who have placed land, glasshouses and labour at their disposal for the experiments, and to Mr. W. E. H. Hodson, A.R.C.S., D.I.C., of the Seale-Hayne Agricultural College for arranging certain duplicate trials in Devon. They are also indebted to Mr. A. Turner, N.D.H., A.R.C.Sc.I., and Mr. H. I. Kingston for the careful way in which the applications were made and the records kept.

Summary of the Life History of *H. radicicola*.

If the tissue of a galled root be broken small white globular bodies can be found; these bodies are the female eelworms or cysts. Microscopic examination reveals that these cysts contain ova and larvæ. In the autumn when the plants are pulled up much of the galled root system is left in the ground and the ripe females perish with the death of the galled tissue. The cysts form a protecting capsule for the eggs and young larvæ and further protection is afforded by the tissue of the galls. As the winter passes into spring the galls gradually decay and liberate many cysts with their contents into the soil, and with the rise in soil temperature in the spring the larval eelworms hatch and wander about in the soil. When susceptible plants are set in such infested soil the young eelworms work their way into the root tissue and derive their nourishment from the plant juices. The sexes mature and mating takes place, followed by reproduction, or reproduction may take place parthenogenetically (5). As development and multiplication goes on the plant tissue presents the localised hypertrophy of the typical galled condition, and the size and extent of the galls increase as eelworm activity becomes more pronounced. Soil moisture and temperature

have an important influence on the eelworms, but they are generally active within the range favourable to good plant growth (6). Since the breaking down of the galls in the soil is a gradual process all the larvæ do not emerge from the overwintering cysts at the same time. This increases the liability of the plants to become infested and renders it possible for plants to become infested so late in the season that they have produced no obvious galls by the time the plants die off in the autumn.

Experiments with Calcium Cyanide.

Calcium cyanide has been fully described by Moore (10). It is made from calcium cyanamide by fusion with sodium chloride in an electric furnace heated to a high temperature. The product is a crude cyanide consisting of calcium, sodium, cyanogen and chloride and is generally considered as having the cyanogen combined with the calcium in the form of calcium cyanide and having a cyanogen content equivalent to 48 per cent. to 50 per cent. sodium cyanide. The commercial form therefore contains calcium cyanide, sodium chloride, carbide, calcium cyanamide and a small amount of sulphur in the form of a sulphide. Calcium cyanide ($Ca(CN)_2$) is quite distinct from calcium cyanamide ($Ca\ CN_2$) in possessing an extra carbon atom and different characteristics. When acted upon by atmospheric or soil moisture calcium cyanide gives off hydrocyanic acid gas whereas with calcium cyanamide no such reaction takes place. In the granular form, as used in the experiments herein described, the crude calcium cyanide is bluish grey in colour and about 90 per cent. passes through a sieve of twenty meshes to the inch and 40 per cent. through a sieve thirty meshes to the inch. In ten samples of the commercial form analysis showed a minimum calcium cyanide content of 46·20 per cent. and a maximum of 53·12 per cent., the average content of the ten samples being 49·1 per cent.

Calcium cyanide in the granular commercial form is dry, it runs freely and when mixed with water it readily dissolves forming a solution of calcium cyanide similar to the solution of sodium cyanide in water and does not give off its hydrocyanic acid gas any more quickly. It is, therefore, especially suitable for work in connection with the control of soil pests.

In dealing with "field trials" for the control of a soil pest such as

Heterodera radicicola mathematical accuracy is scarcely possible and no such claim is made for the results presented herein. The following method was adopted in most cases. Wherever possible the infested greenhouse was examined in the late summer or early autumn while still carrying a crop or at the time when the old plants were being removed, and the general scheme for plot arrangement was decided upon. The plots were arranged to include untreated areas and areas to which varying weights of calcium cyanide either in solution or in dry granular form were applied. When applied in the dry form the cyanide was weighed out into suitable receptacles, tins or paper bags, and allotted to appropriate areas of ground; the cyanide was worked into the soil during trenching operations in preparation for the crop. When applied in solution the cyanide was either mixed with water in a tank and applied with watering cans fitted with roses or by means of a spraying outfit, at a known rate in gallons per square yard. Wherever possible the plots were laid out in duplicate. Plants were set out on the plots in due course and were examined periodically during the growing season, and when the fruit was all gathered the plants were carefully lifted in order to examine the root systems for eelworm infestation. Since these experiments were carried out over two seasons in several localities, it is thought that sufficient data and experience have been accumulated to justify the publication of the results.

Standardization of Results.

In order to have a standard by which to measure the results of the treatment, the typical galled root systems of infested plants were separated out according to the amount of infestation by eelworm as indicated by the amount of galled roots. These root systems were classified as "Free from galls," "Slightly galled," "Moderately galled," and "Badly galled," and then each group was critically examined and the numbers and size of the galls recorded. The results of this examination are given in Table I. When the plants from the various trials were examined this mode of classification was adopted as it appeared to reflect a more or less accurate comparison. Figures 2 and 3 give an idea of the appearance of typical root systems according to the classification; figure 1 shows a plant classified as "Free from galls" (F), and figure 4 a type classified as "Very badly galled" (VB) in some of the tables of results.

TABLE I.

STANDARDIZING DEGREE OF EELWORM INFESTATION.

Classification—Badly Galled. (= B in subsequent Tables.)

LENGTH OF GALLS.

Plant No.	No. of Nodules.	25 mm. or over.	20-25 mm.	10-20mm.	5-10 mm.	Under 5 mm.
1	12	3	0	6	1	2
2	14	3	3	6	2	0
3	21	4	5	6	4	2
4	16	3	2	8	2	1
5	22	4	3	8	5	2
6	13	0	1	5	5	2
7	15	3	3	6	1	2
8	15	4	4	3	3	1
9	15	1	2	4	5	3
10	16	2	2	5	2	1
Average per plant	15·9	2·7	2·5	5·7	3·0	1·6

Classification—Moderately Galled. (= M in subsequent Tables.) (See fig. 2.)

Plant No.	No. of Nodules.	25 mm. or over.	20-25 mm.	10-20 mm.	5-10 mm.	Under 5 mm.
1	7	0	0	4	1	2
2	9	0	0	4	3	2
3	5	0	0	5	0	0
4	6	0	0	4	1	1
5	12	1	0	4	7	0
6	11	0	0	6	3	2
7	8	0	0	4	2	2
8	10	0	0	3	7	0
9	5	0	0	4	1	0
10	7	0	2	1	3	1
Average per plant	8·0	0·1	0·2	3·9	2·8	1·0

Classification—Slightly Galled. (= S in subsequent Tables.) (See fig. 3.)

Plant No.	No. of Nodules.	25 mm. or over.	20-25 mm.	10-20 mm.	5-10 mm.	Under 5 mm.
1	2	0	0	1	1	0
2	1	1	0	0	0	0
3	1	0	0	1	0	0
4	3	2	0	0	1	0
5	2	0	0	0	2	0
6	2	0	0	0	1	1
7	3	0	0	0	2	1
8	1	1	0	0	0	0
9	1	1	0	0	0	0
10	1	1	0	0	0	0
Average per plant	1·7	0·6	0	0·2	0·7	0·2

Experiments on Tomatoes.

Experiments on the control of *Heterodera radicicola* on tomatoes were put down at five centres, but owing to misunderstanding on the part of one of the growers the plants at one centre were pulled up before the final inspection. The results obtained at the other four centres are given fully below.

Series 1. (Lea Valley, 1926). In this series the soil of two glasshouses was treated. Examination of the plants of the previous crop showed that heavy infestation by eelworm was general throughout the houses and that both surface roots and more deeply penetrating roots were badly galled. As was the practice on this nursery the plants were pulled up in the autumn and many roots and galls were left in the soil. The soil, a free working, lightish loam, was prepared by the usual method of removing one spit of top soil, digging the sub-soil, and then turning the next top spit on to this dug sub-soil ; the paths were dug in this way with the rest of the ground. During the digging process many but by no means all of the larger galled roots were removed. A small trench about six inches deep was dug round the base of the walls of the house and the walls were then sprayed with a wash of emulsified cresylic acid, 55 to 60 per cent. pure, at a strength of one in twenty. The beds were divided

into nine plots ; one reserved as a check plot received no calcium cyanide, while the remaining eight received dosages of calcium cyanide varying from 200 lbs. per acre to 2,000 lbs. per acre. The appropriate amount of calcium cyanide was weighed out and applied in the process of digging, some sprinkled on the sub-soil and some worked into the surface soil. The second glasshouse was marked out similarly to the first and the plots received similar dosages. In this house, however, the appropriate amount of cyanide for each plot was dissolved in five gallons of water and applied to the soil when the ground was being dug ; afterwards each plot was

TABLE II.

Plot No.	No. of Plants.	Amount of $Ca(CN)_2$.	Classification.				
			%F.	%S.	%M.	%B.	%VB.
		Dry Method of Application.					
1	28	Nil	—	—	13	42	45
2	49	200 lb. per acre	—	70	26	4	—
3	48	350 ,, ,,	—	21	29	38	12
4	49	500 ,, ,,	—	28	31	38	3
5	38	750 ,, ,,	15	23	35	27	—
6	45	1,000 ,, ,,	22	41	27	6	4
7	45	1,250 ,, ,,	51	37	12	—	—
8	48	1,500 ,, ,,	42	42	16	—	—
9	47	2,000 ,, ,,	83	15	2	—	—
		Wet Method of Application.					
1a	30	Nil	—	7	13	38	42
2a	44	200 lb. per acre	6	25	23	23	23
3a	45	350 ,, ,,	—	25	18	25	32
4a	41	500 ,, ,,	30	31	23	8	8
5a	44	750 ,, ,,	51	26	23	—	—
6a	46	1,000 ,, ,,	76	24	—	—	—
7a	46	1,250 ,, ,,	80	20	—	—	—
8a	48	1,500 ,, ,,	63	37	—	—	—
9a	48	2,000 ,, ,,	89	11	—	—	—

hosed using about one gallon of water to five square feet. The treatment was applied in September, 1925, and the soil left undisturbed until the spring of 1926, when tomato plants were set out on the plots at the usual distance apart, each house accommodating 400 plants. The plants received ordinary cultural treatment during the growing season,

and cropped well, only eleven casualties being recorded from the two houses during the season. Early in August the plots were inspected; the plants on all the plots had made good growth, but the difference in the vigour of the plants on the treated and untreated plots was obvious. Plants on the treated plots had made more vigorous growth, the foliage was a fresher green and the stem stouter. This difference may have been due to the nitrogenous residue accruing from the breaking down of the cyanide in the soil rather than to any checking of the growth of the plants on the untreated plots owing to infestation by eelworm. The plants were still fruiting and by about mid season had yielded an average of about 3 lbs. of tomatoes per plant.

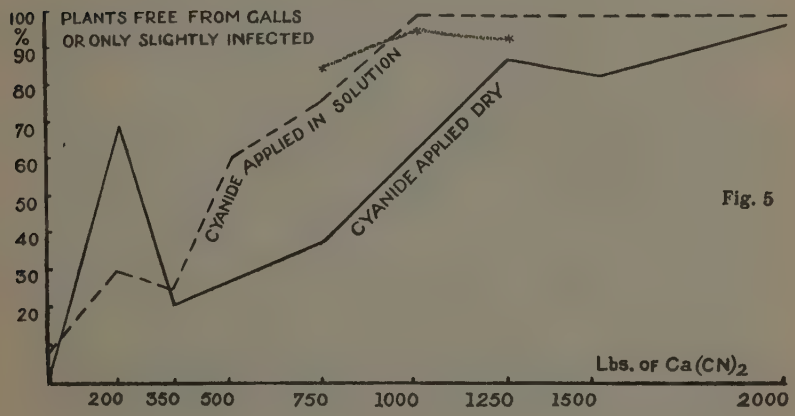

Fig. 5

At the end of September the plants were lifted and the root systems classified according to the amount of galling present. The accompanying graph (fig. 5) and Table II. summarise the experiment and the results obtained, the " a " plots listed in the table being those in the second greenhouse where the wet method of applying the cyanide was employed. Throughout the series the wet method had the advantage,

only slightly with the higher dressings but distinctly with the lower dressings. The influence of the cyanide on the condition of the roots was apparent at the lowest dressing but it was not until the 750 lbs. per acre dressing was reached that reasonable efficiency as regards freedom from galling was obtained. Dressings of 750 to 1,250 lbs. per acre applied by the wet method yielded over 50 per cent. of the plants free from galls. For graphic representation of the degree of control obtained the percentages of plants free from galls (column F) and plants only slightly galled (column S) were totalled. The explanation of the sharp rise at 200 lbs. per acre was not determined but was probably owing to soil influences which might run across both houses since these were parallel.

Series II. (The Lea Valley, 1926).—This series was much less comprehensive than Series I., only four plots and a check plot, each seventeen feet by fourteen feet, being laid down. In the glasshouse used the soil was heavy, retentive and sticky, and when the work of applying the calcium cyanide was commenced on November 1st, 1925, the lower part of the stems and roots of the previous crop were still in the soil. Examination showed that numbers had rotted away, and the remainder were very badly galled and commencing to decay. The soil of the plots was double dug and to plots 1 and 2 the cyanide was applied dry, being spread in a layer on the broken up bottom spit and the top spit worked back over it. To plots 3 and 4 the cyanide was applied in solution to the double dug soil. The work was finished in November. Immediately prior to planting out the crop the grower worked in a dressing of farmyard manure in accordance with his usual custom. The plants were set out in the spring of 1926, each plot accommodating from 94 to 98 plants. At first the plants in the treated plots made good headway and grew away from those on the untreated plots ; later their rate of development slowed up and the plants on the untreated plot soon equalled them for rate of growth and luxuriance of foliage. When inspected at the beginning of August it was at once apparent that little or no benefit had been derived from the calcium cyanide treatment ; many plants had died, others were wilting and yellowing and were obviously in poor condition, many suffering from infestation by fungi including *Colletotrichum tabificum.*

The plots were examined in detail in October and the results obtained, which had been already foreshadowed by the August inspection, are given in Table III. Root rot was prevalent throughout the glasshouse and so many plants had perished that little could be gathered from the state of the roots of the plants that remained. In retrospect it appears that the general conditions were unfavourable for a test of the influence of calcium cyanide on the root knot eelworm, and when compared with the results obtained in Series I. it seems evident that soil conditions play an important part in connection with the treatment of *Heterodera radicicola* by chemical means. Examination the previous autumn had showed that there was a good deal of root rot present and it is possible that the dressing of farmyard manure supplemented by the nitrogenous residue from the cyanide may have predisposed the plants to fungus attack ; in any case it is recognised (1) that farmyard manure is a good medium for the development of the *Colletotrichum* fungus.

TABLE III.

Plot.	No. of plants.	Dosage.	Classification of Plants.					
			F.	S.	M.	B.	Root Rot predominant.	Missing.
1	94	573 lb. per acre dry ...	—	—	—	21	21	52
2	96	1,361 ,, ,, ,, ...	6	9	6	17	48	10
3	98	1,361 ,, ,, wet ...	2	8	19	23	23	23
4	98	573 ,, ,, ,, ...	26	14	9	11	30	8
5	95	Nil	6	16	9	11	25	28

Series III. (Worthing, 1926). The glasshouse in which this series was laid down was fairly heavily infested with *H. radicicola* and in 1925 had carried a much reduced crop. The tomato crop had been followed by chrysanthemums in accordance with the usual custom in the Worthing area, and these were removed a few days before the cyanide treatment was applied in November, 1925. The soil was a medium to light loam on a heavier sub-soil and worked down well during the application of the cyanide ; it was double dug and well broken up to a depth of sixteen inches, and the cyanide applied in solution. Appropriate amounts for each plot were dissolved in water and applied with watering cans

evenly over the plots ; and at the conclusion of the treatment for each plot it was well hosed with water to wash the cyanide down into the soil. In this series the path through the centre of the house was not dug and treated like the adjoining plots. As distinct from the Series I. and II. where the houses were heated for hot-house tomatoes, this house was not heated ; the plants were set out in the middle of May and grown under cold house conditions. When inspected in early August, the plants were fruiting well and little difference could be noted between the plants on the treated and the untreated plots. In early October the plots were critically examined and the roots of the plants classified according to the intensity of the galling, the results being indicated in Table IV.

TABLE IV.

Plot.	No. of plants.	Dressing of $Ca(CN)_2$	Classification of Plants.			
			F.	S.	M.	B.
1	102	Nil	9	51	28	14
2	99	750 lbs. per acre	35	49	15	—
3	99	1,000 ,, ,, ...	36	58	5	—
4	95	1,250 ,, ,, ...	33	51	11	—

The treated plots showed a distinct advantage over the untreated plot producing about four times as many plants free from eelworm galls ; there is little to choose, however, between the different amounts of calcium cyanide as reflected by these results. The 1,000 lb. dosage proved rather more efficient than the 750 lb. dosage but the 1,250 lb. dosage fell off slightly. The figures of the total plants free from and only slightly infested with galls are plotted as percentages in fig. 5, the points being marked with asterisks.

Series IV. (Lea Valley, 1927). In this series five glasshouses all known to be heavily infested with the Root Knot Eelworm were selected for the experiments. The soil was a medium loam, well drained and in good condition for working. The soil in all the houses was double dug and well broken up in the autumn, and lightly worked over periodically during the winter so as to promote the decay of the roots and galls which had been left in the soil from the previous crop. Towards the end of January heat was turned on in the houses in an endeavour

to stimulate the eelworms to activity and induce hatching from the overwintered cysts. At the end of a fortnight, when the soil temperature had reached 46 to 48° F. in the five houses, calcium cyanide was applied at intervals to four of the houses ; the fifth which received no treatment was left as a check. The calcium cyanide was used at the rate of 1,200 lbs. per acre in three houses and 900 lbs. per acre in one house. The treated houses accommodated 325 to 331 tomato plants and the untreated house 264 ; in all the houses most of the plants grew well and yielded well. At the end of September the roots were taken up and examined for galling, and the results of the examination are recorded in Table V.

TABLE V.

House No.	No. of plants.	Amount of $Ca(CN)_2$	Date of Application.	Condition of Roots.				
				F. %	S. %	M. %	B. %	VB. %
1	264	Nil	—	2	18	11	48	21
2	325	900 lb. per acre	February 7th ...	84	9	6	1	—
3	326	1,200 ,, ,,	,, 8th ...	97	2	1	—	—
4	325	1,200 ,, ,,	,, 17th ...	96	3	1	—	—
5	331	1,200 ,, ,,	,, 22nd ...	99	1	—	—	—

These results indicate that under the particular conditions obtaining calcium cyanide yielded a very satisfactory measure of control but the difference in the intensity of galling following the use of calcium cyanide at the rate of 900 lbs. per acre and that following the use of calcium cyanide at the rate of 1,200 lbs. per acre is small.

The results obtained from this series of experiments are particularly interesting in view of the conclusions arrived at by workers at the Lea Valley Research Station in 1920 (9). Experiments there indicated that in general, the use of sterilizing agents against eelworm was more effective when applied in autumn before the soil cooled than when applied in the spring before the soil temperature rose. The high degree of efficiency in the control of eelworm which was reached in the above series appeared to be owing to the soil working down well, more of the galls being decayed by February and consequently liberating more of the cysts, and finally to the raising of the soil temperature stimulating eelworm activity so that in all probability the pest was in a less resistant condition than in the autumn.

Trials on Cucumbers.

In Worthing and the Lea Valley trials on the use of granular calcium cyanide against Root Knot Eelworm on cucumbers were carried out. In both cases houses which had been severely infested with the pest the previous season were chosen, and the calcium cyanide was applied at the same rates and in the same manner as when used against the Root Knot Eelworm on tomatoes. The cucumber beds were composed of soil from new turf and compost, six to eight inches deep, and were laid on the treated soil without receiving any vermicidal treatment. When the cucumber plants were examined at the end of the season it was found that practically every plant was infested more or less severely with the eelworm, and for this reason figures were not taken. The outstanding feature regarding the trials was that, in general, on the plants on the treated plots the galling was not noticeable on the first twelve to eighteen inches of root, but as the roots penetrated into the deeper sub-soil they became badly galled.

The difference in character between the galls on tomato plants and those caused by the same organism on cucumber plants is most marked. On the hard, woody roots of tomatoes the galls are compact in texture, globular or elongate in form, and the hypertrophy does not increase the bulk of root to the extent obtaining on infested cucumbers. The galls on cucumber roots are spongy and open in texture, the globular form is characteristic, and, probably owing to the soft, rapid growth of the plants, the irritation caused by the eelworm produces considerable enlargement of the root tissue.

The observations on the trials of calcium cyanide against eelworm attack on cucumbers indicated that surface treatment of the soil is inadequate for these deeply rooting plants, and that it is necessary to devise a method by which the sub-soil can be efficiently treated in order to obtain a suitable measure of control.

General Discussion.

The experiments on tomatoes at three out of four centres indicated that calcium cyanide at the rate of 750-2,000 lbs. per acre was efficient in reducing the amount of eelworm infestation as indicated by the absence of galls on the root systems of plants grown on the treated plots. Most

successful results followed the use of calcium cyanide in solution when applied in the autumn (Series I.) and a high degree of efficiency was obtained by the use of dry, granular calcium cyanide in the spring after the soil temperature had been raised to 46°–48° F.

Soil conditions are important from the point of view of temperature and moisture, for in the case of Series II. the application of the cyanide to a heavy, retentive soil, in the presence of fungus diseases, resulted in complete failure to control the eelworm. With well drained, well broken up, lightish, loamy soil and a satisfactory temperature, calcium cyanide at the rate of 900 lbs. to 1,200 lbs. per acre yielded promising results, producing from 84 per cent. to 99 per cent. of plants free from eelworm galls.

Experiments on cucumbers indicated that since the roots penetrate very deeply into the sub-soil, they soon extend beyond the range of influence of the cyanide and may become infested with eelworms at the lower levels and produce typical eelworm galls towards the extremities of the roots. From this it would appear that when the cyanide is applied in the manner adopted in these experiments, its influence is limited to the surface soil ; and that, though surface rooting plants may be protected from the ravages of eelworm, the more deeply rooting plants need a more complicated method of treatment, probably entailing the trenching of the soil to a considerable depth. This observation bears out the findings of Thorne (16) working on calcium cyanide in relation to the control of *Heterodera schachtii* Schmidt on sugar beet in America. He found that where calcium cyanide was disced and harrowed at the rate of 800 lbs. to 1,600 lbs. per acre all the eelworms to a depth of eight inches had been killed, while below fourteen inches none had been killed. Where the application of 800 lbs. per acre was ploughed in to a depth of ten to twelve inches soil examination showed that practically all the eelworms to a depth of 14 inches were killed, but below eighteen inches the eelworms were not affected. It would seem therefore that success is most likely to be obtained when the cyanide is well incorporated with the soil and worked to a depth approximately equal to that penetrated by the roots of the crop. In this connection the value of concrete bases (2) for cucumber beds might be pointed out, for the roots of the plants could in this way be restricted to soil which was well treated before use.

As a general rule paths in glasshouses should be dug and treated like the rest of the soil, and the soil at the bases of the walls should also be thoroughly treated, if possible so that the treatment would penetrate all crevices of the brickwork ; this is important for in some of the experiments herein discussed it was found that in plots producing a high percentage of clean plants infested plants occurred beside the walls. Since infested root systems are difficult to pull up without breaking, the practice of pulling the old plants out is deplorable. The plants lift out with the aid of a fork with comparative ease, and in this way the bulk of the infested root systems can be removed and burnt, thus reducing the sources of infection for the following crop. Infection is readily carried on tools, wheels, boxes and boots, and these are means of spread to be guarded against for they become of importance when efforts are made to control the pest. Among the principal sources of infestation are soil at the roots of young plants purchased to " fill up " and soil purchased for seed beds and obtained from infested nurseries.

The cost of using granular calcium cyanide at the rate of 1,000 lbs. per acre, exclusive of labour, works out at approximately £55 per acre. This compares favourably with steam sterilizing which costs about £200 per acre, but which has, of course, a considerably wider scope ; and also with cresylic acid, a widely used sterilizing agent, the cost of which is about £45 8s. per acre (3).

Summary.

The Root Knot disease of tomatoes and cucumbers, caused by the attacks of *Heterodera radicicola* Müll., is of widespread occurrence and under certain conditions, notably in the presence of fungi, may cause serious losses. Under some conditions considerable infestation may be present and yet produce no apparent ill-effects on the plants.

Experiments having indicated that sodium cyanide was beneficial in reducing the amount of eelworm infestation and pot trials having suggested that calcium cyanide might also prove effective, trials with calcium cyanide were carried out at various centres in 1926 and 1927.

Where tomatoes were grown in soil treated with 750 lbs. to 2,000 lbs. of calcium cyanide per acre the amount of eelworm infestation, as measured by the amount of root knot, was substantially reduced, over 90 per cent. clean plants being produced in some instances.

The application of calcium cyanide in solution in the autumn proved more efficient than the application of the dry granular form at the same season, but used in spring when the soil temperature had risen to 46° F. to 48° F., 96 per cent. to 99 per cent. of clean plants resulted from the use of the dry form at the rate of 1,200 lbs. per acre.

Efficiency was increased by working the soil thoroughly to promote the decay of the root galls left from the previous crop before the application of the cyanide. Attacked plants should be lifted with a fork at the end of the growing season, instead of being roughly pulled up, for the latter method results in the bulk of the root galls being left behind in the soil to furnish a source of infestation for the succeeding crop.

When soil for cucumbers was treated it was found that the influence of the cyanide did not penetrate far into the base, and when the roots grew beyond the range of influence of the cyanide they became badly infested with root knot.

Explanation of Plate.

Root Systems of Tomato Plants grown in soil infested with the Root Knot Eelworm.

Figure 1.—Roots free from galls—" F " in tables of results.

Figure 2.—Roots moderately galled—" M " in tables of results.

Figure 3.—Roots slightly galled—" S " in tables of results.

Figure 4.—Roots very badly attacked by the Root Knot Eelworm—" VB " in tables of results.

[*Photographs by H. I. Kingston.*]

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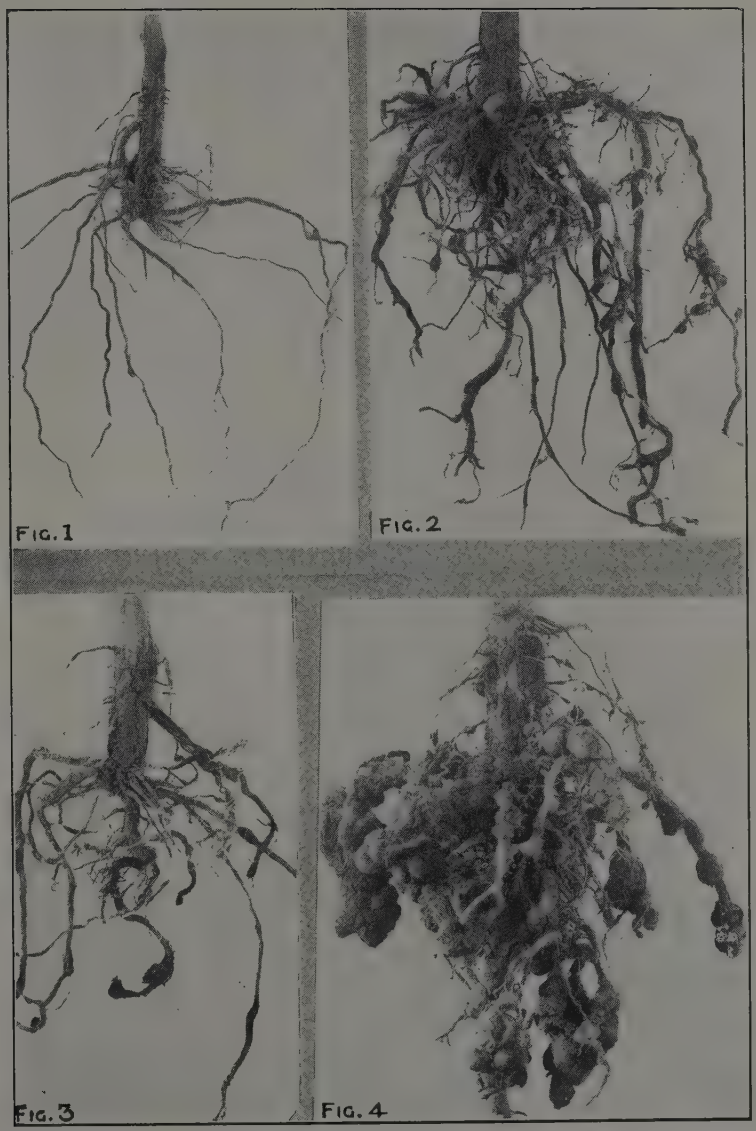

Root Systems of Tomato Plants infected by *Heterodera radicicola* Müller.

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Journal of Helminthology, Vol. VI, *No.* 2, *June*, 1928, *pp.* 77-78.

Sur l'identité des espèces *Rhabditis longistoma* Stefanski, 1922 et *Cylindrogaster coprophaga* Goodey, 1927.

par Witold Stefanski.

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En 1927 Journal of Helminthology a publié une note de Goodey sur *Cylindrogaster coprophaga* gen. et sp. nov., provenant d'une culture des excréments de rat (*Rattus norvegicus*). La note donne une description détaillée de la nouvelle espèce, accompagnée d'excéllentes figures.

Or il réssort clairement de la comparaison de la description ainsi que les dessins donnés par Goodey avec l'espèce décrite par moi en 1922 sous le nom *Rhabditis longistoma* que les deux formes sont identiques. Mr. le Dr. T. Goodey auquel j'ai envoyé mon travail, après avoir prendre connaisance de sa publication tient également pour probable la synonymie de nos deux espèces, m'invitant en même temps à publier une note concernant cette question. Je saisis cette aimable proposition avec d'autant plus de plaisir que mon travail fut publié dans un périodique qui parait être peu répandu à l'étranger.

La description de *Rhabditis longistoma* fut basée sur deux exemplaires dont un mâle et une femelle sur lesquels je poursuivais les expériences concernant les phénomènes de l'excrétion chez les Nématodes libres, c'est pourquoi l'observation des caractères spécifiques n'a pu être ni aussi exacte ni aussi détaillée que celle de Goodey.

Néanmoins l'espèce est tellement caractéristique que son identification ne présente point de difficulté.

1. La description de *Cylindrogaster coprophaga* commence ainsi (Goodey). " The principal distinguishing features of the genus are the long pharyngeal rods " et je lis dans mon texte (1922, p. 12)—" La cavité buccale est très caractéristique pour l'espèce. Elle est cylindrique et sa longeur atteint un tiers de la longeur de l'œsophage." D'après Goodey sa longeur absolue est de 0·038-0·05 mm., c'est qui représente en effet à peu près le tiers de la longeur de l'œsophage.

2. En outre les dessins, No. 3. de Goodey et le mien No. 22, étant tout à fait comparables, on peut s'en rendre compte que la lumière de

la cavité buccale est très étroite et que sa couche cuticulaire commence et se termine par des épaississements.

3. Le bulbe antérieur est non moins caractéristique. Il est cylindrique et sa longeur égale celle de la cavité buccale.

4. Le rétrécissement brusque de l'œsophage formant un col entre les deux bulbes est également indiqué dans les deux textes.

5. La disposition des papilles boursales chez le mâle est identique chez les deux formes. L'observation plus détaillée de Goodey a permis, il est vrai, à l'auteur de reveler l'existence des papilles submédianes, omises dans mon dessin, mais par contre le groupement des papilles boursales reste sensiblement le même. On en distingue en effet dans les deux dessins quatre groupes ; dont le premier, situé assez haut, en avant du spicule, le second, au niveau de son extrémité proximale et le troisième immédiatement en arrière de l'anus, chacun de trois groupes étant composé d'une papille et enfin le quatrième groupe, composé des trois papilles, se trouve à proximité de l'endroit où la queue se rétrécit brusquement.

6. La forme de celle-ci quoique ne réprésentée sur mon dessin qu'en partie montre le rétrécissement caractéristique pour les deux formes.

7. La seule différence importante concernerait la forme du spicule mais mon dessin étant très schématisé cette différence doit être attribuée à l'insuffisance d'observation. Les spicules des deux espèces sont en tout cas minces, le caractère qui n'est pas sans importance dans le groupe de *Rhabditidæ*.

Mon espèce fut trouvé dans le fumier aux environs de Genève. Sa découverte par Goodey dans les excréments du rat confirme son habitat coprophage.

En résumé, je considère *Cylindrogaster coprophaga* comme espèce synonyme de *Rhabditis longistoma* et son nom doit être corrigé ainsi—*Cylindrogaster longistoma* (Stefanski, 1922) Stefanski, 1928.

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Journal of Helminthology, Vol. VI, *No.* 2, *June*, 1928, *pp.* 79-86.

Parastrongyloides winchesi gen. et sp. nov. A Remarkable New Nematode Parasite of the Mole and the Shrew.

By D. O. Morgan, M.Sc., Ph.D.

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Introduction.

The species of the Nematode genus Strongyloides are of considerable interest to helminthologists and extensive studies on the biology of their life-history have been carried out by various workers. Their chief interest lies in the fact that the parasitic stage consists of hermaphroditic forms only, while in the free-living stage there may be a differentiation into males and females before reaching the infective stage. This alternation in the life-cycle is also exhibited by the allied genus Rhabdias, with the exception that, according to Railliet (1899) and Goodey (1922), certain species from the snake do not appear to have this differentiation in the free-living stage.

Among the more recent publications on the life-cycle in the genus Strongyloides is a paper by Sandground (1926) in which a valuable contribution is made to our knowledge of the different modes of development exhibited by some of the species of this genus. In addition he shews that the parasitic female is not parthenogenetic as it was previously held to be, but hermaphroditic.

The present paper does not deal with the problems directly connected with the life-history of these species; it is, however, considered to be of much interest in that it records for the first time the finding of a parasite, closely resembling Strongyloides, which is quite unique in having a male in the parasitic generation. For reasons which will

be given later in the paper the writer has decided that the erection of a new genus to include this species is desirable, and the name Parastrongyloides gen. nov. is selected to shew its close relationship to Strongyloides, with *winchesi* sp. nov. from the mole (*Talpa europæa*) as the type species.

The parasites were obtained from the intestine of moles caught on the experimental farm of the Institute of Agricultural Parasitology. Some fifteen moles were examined and at least half of these contained this species. Only three or four worms were usually present and females were found more frequently than males. This may have been due to the fact that the worms are very small and slender and are consequently overlooked quite easily even when the intestinal contents are subjected to a very close scrutiny under a binocular microscope. As far as the writer is aware no species of Strongyloides has been recorded from the mole and it is somewhat surprising that the new species described below has not been met with before. Its small size would probably account for this.

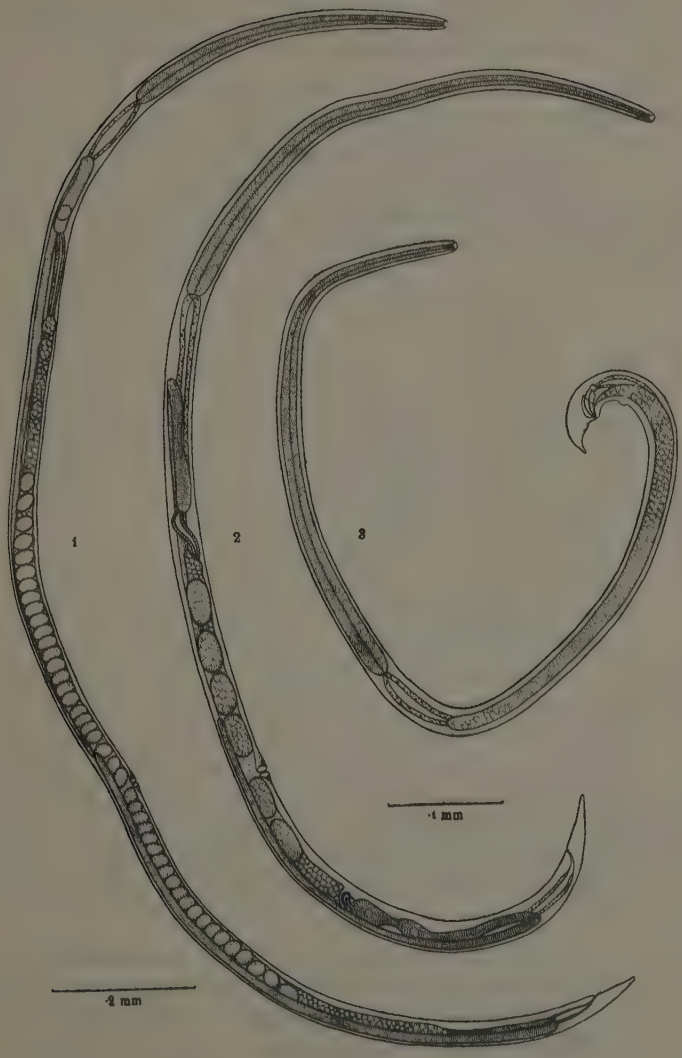
Technique.

The worms were collected and fixed in hot Ditlevsen's fixative, as given by Thorne (1925). They were then transferred to a mixture of 30 per cent. alcohol and 2 per cent. glycerine which was allowed to evaporate slowly at laboratory temperatures until a medium of almost pure glycerine was reached. The worms were then mounted in this medium and examined. It was found that this method gave slightly better results for small forms than the usual one of fixing in hot glycerine alcohol.

Morphology.

The parasites are very slender and similar in general appearance to a typical Strongyloides species. The cuticle appears to be smooth except for some faintly defined longitudinal striations. Even under the highest powers of the microscope no trace of transverse striations

Figs. 1, 2 and 3.—Two females and a male of *Parastrongyloides winchesi* drawn under low magnification to show the principal features of the anatomy. Figures 2 and 3 are drawn to the same scale.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were observed and in this respect it differs from Strongyloides species. The mouth is terminal and in some of the specimens there were indications of lips, six in number, and conical in shape. No trace of papillæ was observed.

Alimentary Canal.—The mouth opens into a short but distinct vestibule which is cup-shaped, has slightly thickened walls and is similar to that found in species of the genus Rhabdias. The œsophagus is long like that of a typical species of Strongyloides. Its length averages 0·5 mm. ranging from 0·43 mm. to 0·54 mm. The anterior half is narrow with a width of about 0·01 mm. whereas the posterior half fills the greater part of the width of the body. It is thought that the position of the nerve cord and possibly the excretory pore is situated about the point where the œsophagus widens out, but a very close study of a number of specimens failed to reveal this, with any certainty. The variation in the length of the œsophagus does not appear to depend on the length of the worm and it was found that there was very little difference in this respect between the smaller and the larger group of females. The œsophagus opens into the intestine which terminates in a short and rather indistinct rectum.

Male.—The average length of the male is 1 mm. and the width ranges from 0·015 mm. at the head end to 0·04 mm. in the region of the spicules. The œsophagus is about 0·46 mm. in length and exhibits almost the same range of variation as that of the female. The tail is bent inwards towards the ventral surface just as we find in the males of Strongyloides species. It measures 0·04 mm., is short and blunt and therefore unlike the long pointed tail of the latter. There is a pair of post-anal papillæ situated near the mid-ventral line and also one pre-anal, which gives the appearance of a small flat pad on the ventral surface. The writer was unable to obtain a ventral view of this papilla ; its lateral aspect, however, was somewhat suggestive of a small ventral sucker corresponding to that found in members of the Heterakidæ.

Both the spicules and the accessory piece appear to be identical with those found in the free-living males of Strongyloides as figured by Goodey (1926) for *S. stercoralis* and *S. fülleborni*. The former are shaped like a curved knife with a knob-like head followed by a short constriction ; the remaining portion broadens out like a knife blade. They are thickened

along three curved ridges which run from the tip into the knob-like head. The spicules measure approximately 0·04 mm. The accessory piece is shorter and is shaped like a short-handled scoop enfolding the spicules.

The male gonad extends forward almost as far as the base of the œsophagus and for the greater part of its length occupies most of the width of the body. It does not appear to be reflexed at its anterior end.

Female.—Considerable variation in the total length of the females was obtained, in fact the specimens examined fell into two distinct groups. One group contained worms with an average length of 1·46 mm.

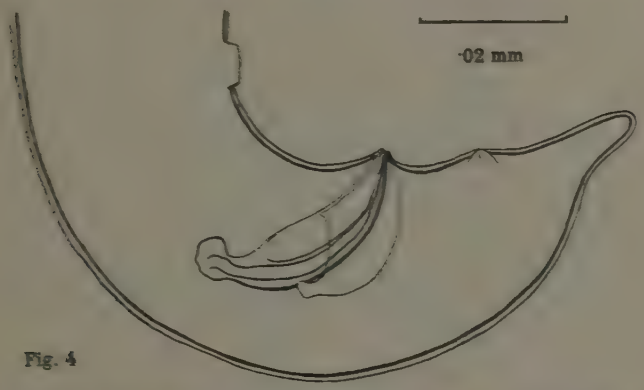

Fig. 4

Fig. 4.—Tail end of male of *Parastrongyloides winckesi*, in lateral view, highly magnified, shewing a spicule, the accessory piece and the caudal papillæ.

exhibiting a range of 1·2 mm. to 1·6 mm. and containing only a few eggs, usually less than ten. The second group of three females gave an average measurement of 2·2 mm. and contained a large number of eggs, in one specimen these numbered over forty. Figures 1 and 2 bring out this difference.

The breadth of the female at the anterior end is 0·01 mm.; 0·035 mm. in the region of the vulva and 0·02 mm. at the anus. The tail is short and blunt and measures 0·05 mm. from the anus to the tip.

The vulva is situated on the ventral surface of the body at a distance of about 0·5 mm. from the tail in the smaller specimens,

and about 0·9 mm. from the tail in the larger specimens. Its position can therefore be said to be in the region of the posterior third of the body and in this respect similar to the females of Strongyloides. The lips of the vulva are prominent and slightly raised and are formed by a thickening of the body wall in this region. The vulva opens directly into a uterus composed of two opposed branches which become reflexed. Distally the opposed limbs meet again at a point a little anterior to the vulva. This, however, is not invariably the case and in some specimens the two branches did not meet while a further folding of the gonad was observed in one instance. The first portion of the gonad near the vulva consists of the uterus containing eggs in an advanced stage of segmentation. This is followed by a distinct receptaculum seminis containing spermatozoa, its position and size varying somewhat and probably depending on the number of eggs in the uterus. The receptaculum seminis is not continued directly into the next region of the gonad but ends as a blind sac. Its tubular connection with the ovarian part is by means of a narrow, thick-walled and often coiled duct, which issues from the receptaculum seminis just a little short of the blind end. Goodey (1924) describes a similar duct in *Rhabdias fuscovenosa* and also suggests that it functions as a shell gland. This duct passes distally into a portion containing unsegmented eggs which then becomes reflexed and runs towards the region of the vulva.

The eggs are elliptical in shape and those situated near the vulva appear to be in an advanced state of segmentation. They are somewhat small in size having an average measurement of 0·04 mm. by 0·02 mm.

Parastrongyloides winchesi in the Shrew.

A few specimens of this genus were obtained in one instance from a shrew and a casual study of these at first suggested that they might belong to a new species. It was found, however, after making a more detailed study that there were no true morphological differences upon which they might be separated from *P. winchesi* described above. Slight differences in measurements were obtained, particularly in the length, of the œsophagus. The latter was found to be 0·39 mm. in the female and 0·36 mm. in the male as compared with 0·5 mm. and 0·46 mm.

respectively in the specimens from the mole. The width of the body was, on the whole, greater in the former than in the latter specimens. One would point out, however, that some disintegration of the cuticle had set in in the shrew material and consequently when mounted under a coverslip the worms tended to become flattened out more than normally.

The writer considers that the variations in size hardly justify the making of a new species for the parasites found in the shrew. It is hoped to be able to make a more detailed study of the morphology of this form when fresher material can be obtained. For the present the shrew is added to the mole as a host for *Parastrongyloides winchesi*.

General Remarks.

Parastrongyloides winchesi differs from Strongyloides species in having a distinct vestibule and in this respect resembles the genus Rhabdias. There are no other differences in the female if we except the well defined receptaculum seminis in which it again resembles the genus Rhabdias. In comparing the parasitic male of *P. winchesi* with the free-living male of a Strongyloides species we find the obvious difference in the length and shape of the œsophagus and also in the shape of the tail; that of the former is short and blunt and the latter long and pointed. Again the post-anal papillæ are ventral in *P. winchesi* and sub-dorsal in Strongyloides species. This comparison is, however, hardly justifiable since we are comparing the parasitic male on the one hand with a free-living male on the other.

It will be seen, therefore, that the new species differs very little from Strongyloides species and on morphological grounds it could be included in this genus. If included in Strongyloides it would, however, stand apart very distinctly from all the other species. In addition, one finds in *P. winchesi* the existence of a male in the parasitic generation and in view of this the writer considers it avisable, for the present at any rate, to erect a new genus to include this species. This will tend to avoid confusion by retaining Strongyloides as a compact genus having only the female hermaphroditic form in the parasitic generation.

The writer has so far been unable to obtain a sufficient quantity of material at any one time to carry out experiments on the larval development of *P. winchesi*. It would undoubtedly be of considerable interest

if the infective larva of *P. winchesi* should prove to be of the unsheathed filariform type like that of Strongyloides species and also if males and females should occur in the free-living generation.

The possible existence of parasitic males in some species, both of Strongyloides and Rhabdias can no longer be overlooked, particularly so in certain species of the latter genus from snakes which do not appear to be differentiated into males and females in the free-living stage and which show a well defined receptaculum seminis in the parasitic female.

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On Some Parasites of the Rusty Tiger Cat (*Felis planiceps*).

By Thomas W. M. Cameron, M.A., Ph.D., D.Sc., M.R.C.V.S.

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In October, 1927, a rusty tiger cat, *Felis planiceps*, died at the Zoological Gardens, London, three weeks after imporation from the Malay States. An examination of the intestinal tract and of certain portions of the connective tissue revealed the presence of at least a dozen different species of helminths, embracing many diverse groups of worms. In the connective tissue, in addition, were a number of small oval cysts, somewhat resembling a trichina cyst in outline, but much larger in size and with a totally different structure. Fat globules were seen at the poles. It was not found possible to identify them but it is possible that they were parasitic in origin.

TREMATODA.

Strigeidæ.

A number of specimens of *Alaria* sp. were recovered from the small intestine but on account of their poor state of preservation, the species could not be determined.

Dicrocoeliidæ.

About half a dozen flukes belonging to the genus *Platynosomum* were found in the small intestine. The liver was examined by Dr. H. H. Scott, who performed the autopsy, and appeared to be normal, although it was not specially examined for the presence of helminths.

Platynosomum planicipitis sp. nov.

The total length of the largest specimen was only 2.4 mm. (fig. 1), with a maximum breadth of 0.65 mm. The outline of the body is lanceolate.

The oral sucker is terminal with a diameter of 0·2 mm. The ventral sucker is at the junction of the anterior and second quarter of the body. It is large and prominent with a diameter of 0·33 mm. The oral sucker opens into a short œsophagus which is surrounded at its origin by a small pharynx. The œsophagus bifurcates about mid-way between the oral and ventral suckers and the cæca terminate a short distance from the posterior end of the body.

The testes lie side by side just behind the ventral sucker. They are large spherical bodies, 0·2 mm. in diameter and are separated from each other by a single loop of the uterus. The cirrus is a prominent object in front of the ventral sucker. Close behind it is the seminal vesicle. The genital pore is about the level of the bifurcation of the intestine.

The ovary lies just posterior to one—usually the left—testis. It is spherical with a diameter of 0·1 mm. Just behind it is the shell gland. There is no receptacle seminalis. The yolk glands occupy two fan-shaped masses lateral to the intestinal branches and situated about the middle of the body. The yolk ducts leave the glands at the apex of the fan and join just posterior to the shell gland. The uterus is dendritic, pursuing a winding course to the posterior end of the body. It loops anterior and lateral to each mass of yolk glands, passes between the testes, and after coiling behind the ventral sucker opens at the level of the bifurcation of the gut. The ova are oral and have an average size of 32·5μ by 21μ.

In 1901, Braun described from *Viverra zibethi* a species of Platynosomum which he named *Dicrocoelium concinnum*. This species is 2·7 mm. to 3·3 mm. long with a maximum width (at the level of the yolk glands) of 1·6 mm. long. The body is egg-shaped, the posterior end being round and broad. The yolk glands form two fan-shaped masses at the level of the ovary, just posterior to the middle of the body. The ova measure 41μ to 45μ long by 23·3μ broad.

In 1910 Kossack described from *Felis minuta* another species of *Platynosomum* (viz. *P. fastosum*) and Ware re-described the same species in 1923, from the domestic cat (from Malay States and Dutch and British Guiana). This species varied from 4·59 mm. to 7·5 mm. long and 1·5 mm. to 2·5 mm. broad. The body is lanceolate with lateral

margins parallel for a good deal of their length. The yolk glands occupy a longitudinal area (about one-fifth of the body length) and are *not* fan-shaped. The ova are 34μ to 40μ by 21μ to 25μ. No other species of *Platynosomum* appears to have been described from carnivorous animals.

The present species has a total length of about 2·4 mm. and a breadth of about 0·65 mm. The body is lanceolate in shape, the posterior extremity being bluntly pointed. The yolk glands form two roughly

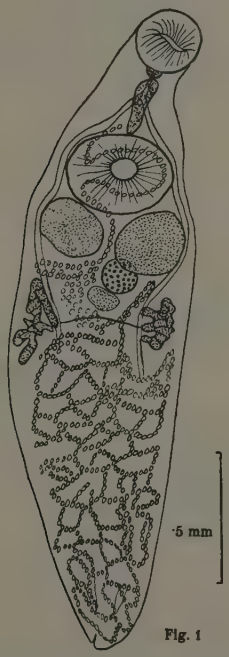


Fig. 1

Platynosomum planicipitis sp. nov.

fan-shaped masses at the middle of the body. The ova measure about 32·5μ long by 21μ broad.

It will be seen, therefore, that the species is intermediate between the two older forms. It is similar in size and shape of the yolk glands to *P. concinnum;* and in shape and size of the ova to *P. fastosum*. In other respects the three species are similar.

It is accordingly proposed that this form should be recognised as a new species—*Platynosomum planicipitis*—closely related to *P. concinnum*. It is possible, however, that subsequent work may make it necessary to regard it as a synonym of the latter. The shape of *P. concinnum* is so characteristic—and Braun lays emphasis on the shape—that the writer does not consider himself justified at present in regarding it as the same species.

CESTODA.

Two larval cestodes were found which I have not been able to identify definitely. They are elongated cysticerci with an invagination at the anterior end. At the bottom of this is an inverted scolex with four suckers but no hooks. The invagination is very small compared with the length of the cyst. The length of the cyst is from half to one centimeter and the breadth is about one and a half millimeters. The lateral edges are crenated and the posterior end has a small indentation.

Hymenolepidæ.

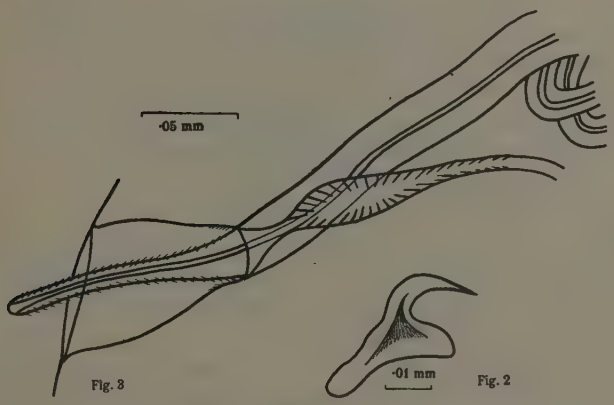
In addition to these larval stages, a number of specimens of a single species of adult tapeworm was recovered from the small intestine in a good state of preservation. It appears to represent a new genus.

Ælurotænia planicipitis gen. et sp. nov.

This species has about 180 to 200 segments and its maximum length is 35mm.; the maximum breadth (of expanded segments) is 0·6 mm. The scolex is about 0·3 mm. in diameter and is pyriform in shape. The four suckers have a diameter each of 0·1 mm. There is a large rostellum present and a single circlet of about 12 hooks. These hooks are all about the same size (fig. 2) and measure (in a straight line from the tip of the blade to the end of the handle) 0.36 mm. The blade is sharply curved and it joins the guard at right angles. The tip of the blade is accordingly parallel to or even approaching the guard. The guard is blunt, cylindrical and spherical in cross section. The handle is relatively long and undulating. Its upper surface has a "hump" at its junction with the blade, while its lower surface forms a uniform arc with the guard. There is a long neck and some 120 segments occur between this and the fully mature segments, of which there are only sixteen to twenty. The immature segments are much broader than long. This ratio gradually

decreases until in the fully gravid segments, the breadth equals the length (in relaxed specimens) while in the case of the last few segments it is even less than the length. Each proglottid slightly overlaps the succeeding. The segments have a very strongly developed double inner layer of longitudinal muscles arranged around the genitalia. The subcuticular longitudinal layer as well as the transverse muscles are only poorly developed.

The excretory system consists of two pairs of longitudinal canals. The smaller dorsal canals do not appear to extend beyond the mature segments, but the large ventral vessels continue to the posterior end of the segments.



Ælurotænia planicipitis gen. et sp. nov.

Fig. 2.—Hook. Fig. 3.—Genital opening shewing cirrus, vas deferens and vagina.

The genital pores are situated in the anterior portion of each segment and are unilateral. Both male and female ducts pass ventral to the ventral excretory vessel. These ducts open side by side, the female ventral to the male. Their course is parallel for a considerable portion of their length. They both run anteriorly to the extreme top of the segment. The male canal forms a very complicated coil at this position, while the female canal is directed posteriorly towards the female glands (figs. 3 and 4).

The Male Organs.

An elongated cirrus is present, covered with numerous minute spines, all of the same size. This is continued as a simple vas deferens as far as the middle anterior field when it is thrown into numerous folds. There is no seminal vesicle present.

There are about 15 testes present, each measuring about 0·025 mm. in diameter. These lie in a single dorso-lateral layer in the centre of the segment grouped around the female glands and crossing the middle line posterior to them. There are no testes present in the middle line anterior to the ovary.

The Female Organs.

The course of the vagina has been described above. The vagina dilates immediately after leaving the vulva and is lined by a prominent layer of what appear to be glands. This dilation gradually decreases and the remainder of the tube has a uniform diameter.

The ovary consists of two kidney-shaped bodies connected at the anterior end by a commissure. Between the posterior ends lies the single spherical vitalline gland.

The uterus commences as a double sac lying above the ovary, the two portions of which coalesce to form a single kidney-shaped organ, the hilum of which is posterior. This increases in size as it fills with ova and gradually become irregular in outline owing to the inward growth of fibrous tissue. Finally, when the uterus has come to occupy the major part of the segment, these fibrous strands have broken it up into a large number of egg capsules, each containing as a rule a single egg—occasionally more.

The ova are spherical to oval in shape, 30μ to 40μ in diameter; the shell is thin and hyaline.

Following Meggitt's classification, this species would seem to belong to the sub-family Dipylidiinæ of the family Hymenolepidæ, being closely related to the genus *Similuncinus*.

This genus was created by Johnston in 1909 to receive a cestode found in the Laughing Jackass (*Dacelo gigas*). It resembles the present species in many points but differs from it in others. There are thirty to thirty-six hooks with a sharply pointed handle with which the blade is continuous. There are fifty to sixty testes and the vas deferens coils just before and after

entering the cirrus sac—not in the middle field. There is a distinct seminal receptacle present; the ovary is small, not distinctly bilobed, while the yolk gland is bilobed. The uterus is reticulated (resembling that of *Dipylidium*), the diverticula surrounding the testes and ultimately forming egg-nests. In the present species there are only 12 hooks with a blunt handle and with the blade set at an angle to it. There are about 15 testes and the vas deferens coils only in the middle field. There is no distinct seminal vesicle, the ovary is bilobed while the yolk gland is spherical. The uterus is sac-like, only secondarily forming egg-capsules by the ingrowth of connective tissue.

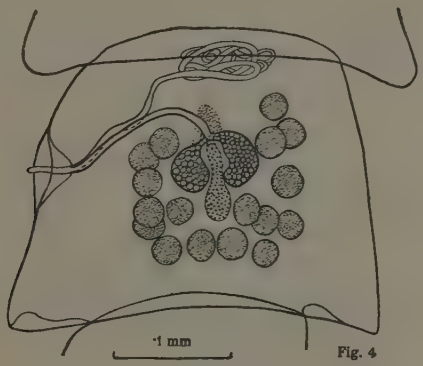


Fig. 4

Ælurotænia planicipitis gen. et sp. nov.

Fig. 4.—Mature proglottid.

These differences seem to be sufficiently pronounced to justify the formation of a new genus *Ælurotænia*, with the new species *Æ. planicipitis* as type.

NEMATODA.

Rhabditidæ.

Strongyloides.—A single specimen of Strongyloides was found. Its total length was 1·8 mm. The œsophagus is 0·65 mm. long with a maximum breadth of 0·025 mm. The anus is situated 0·05 mm. from the tip of the tail, which is narrow and bluntly pointed. The maximum breadth of the body is 0·2 mm. at the level of the vulva. The breadth

at the level of the anus is 0·01 mm. while the breadth of the tail, which is almost uniform in thickness, is 0·005 mm. The vulva is situated 0·7 mm. from the tip of the tail, and therefore divides the body in the ratio of 11 : 7.

This specimen is shorter and narrower than *Strongyloides felis* as found by Chandler in cats in Calcutta. The tail is shorter and the vulva slightly farther forward. On the other hand, the œsophagus is longer in proportion to the body length. The tail has parallel sides and has a blunt tip, while Chandler's species has a tail, the sides of which evenly taper to a sharp point.

These differences may be only individual, as only a single specimen was available for examination. Accordingly, while it does not seem advisable to refer it definitely to *S. felis*, a new name is not proposed for it until other specimens from cats in Malay have been examined.

Ascaridæ.

Belascaris mystax.—A single male of this species was found in the small intestine, together with a number of larvæ of the same species.

Porrocæcum sp.—A single immature female Porrocæcum was found in the stomach. It was in a good state of preservation and was probably alive at the time of the death of the cat. The cat had been in the Zoological Gardens for three weeks, during which it had been fed on sparrows, but on no other birds or fish.

Filariidæ.

In the connective tissue of the hind leg was a single female *Dirofilaria*, but no male was found. In the same situation were fragments of another filaria which was much thinner, and which, although too degenerate to identify, probably belongs to a different genus.

Spiruridæ.

In the mesentery were two encysted larvæ of *Gnathostoma* which correspond in their morphology with those described by Chandler (1925) from snakes in India.

In addition, a single encysted larva of another species of Spirurid was found, but was too immature to identify.

Ancylostomidæ.

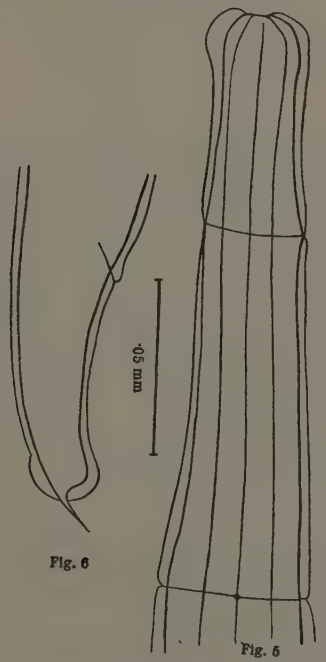
A number of specimens of both sexes of *Ancylostoma braziliense* were

found in the small intestine. This was the only species of hookworm present.

TRICHOSTRONGYLIDÆ.

Nematostrongylus planicipitis gen. et sp. nov.

Only a single male and female of this minute trichostrongyle worm were found. The male has a total length of 4·35 mm. with a maximum breadth (just anterior to the bursa) of 0·06 mm. The female is 7·75mm. long with a maximum breadth (at the level of the vulva) of 0·12 mm. The skin has very fine transverse striations.



Nematostrongylus planicipitis gen. et sp. nov.
Fig. 5.—Head. Fig. 6.—Tail of female.

The Head End (fig. 5).

The cuticle surrounding the simple mouth is dilated slightly, in a manner similar to *Microstrongylus genettæ*: and as in that species a cervical ridge and an excretory groove are present. The cervical ridge

is situated at the junction of the anterior and second sixth of the œsophagus. It consists of a slightly raised portion of body substance, on the surface of which the cuticle is abnormally thin. The excretory pore is situated about the level of the junction of the second and third fifths of the œsophagus. It lies in a groove, which like the cervical ring, completely encircles the body. This groove, however, is much shallower on the dorsal than on the ventral surfaces and the thickening of the cuticle is much less marked than in *Microstrongylus*.

The œsophagus is only slightly swollen posteriorly ; it is about 0·4 mm. long with a maximum breadth of 0·024 mm.

The Female.

The anus is situated 0·065 mm. from the posterior end, which is slightly swollen, and through the cuticle of which projects a spike of body substance (fig. 6). The anal opening is the usual transverse slit. The rectum and intestine present no special features. The vulva also is a transverse slit situated in the posterior part of the body, which it divides in the ratio of 4 : 1. The ovejectors are of the typical Trichostrongyle type. It is about 0·25 mm. in length and the two ovejectors are equal in size. The genital tubes diverge. The posterior tube runs to a short distance anterior to the anus, turns, and twisting on itself, terminates some distance in front of the ovejectors. The anterior tube turns some distance behind the œsophagus, and, twisting on itself, terminates just anterior of the other tube. No fully formed ova were seen.

The male bursa (fig. 7) when spread out has a breadth of 0·16 mm. It is very similar to that of *Microstrongylus*. The externo-dorsal ray, however, is shorter and stouter. The terminations of the dorsal ray also differ slightly and do not present the typical " trident "arrangement seen in that genus.

Small prebursal papillæ are present.

The spicules are equal and similar and are relatively short and stumpy. Each terminates in three sharp points, of which the middle is needle-like and the outer two comparatively stout. The spicules are 0·08 mm. long. The accessory piece is 0·06 mm. long and has an undulating

outline without the posterior keel seen in *Microstrongylus*. It has a fish-hooklike posterior end and a bluntly pointed anterior end.

This is the third species of trichostrongyle worm to be described from

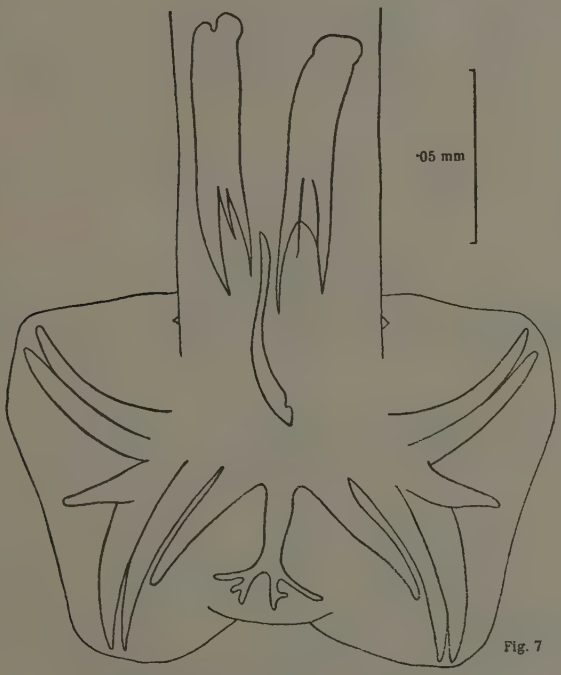


Fig. 7

Nematostrongylus planicipitis gen. et sp. nov.
Fig. 7.—Bursa and spicules of male.

Carnivores, the others being *Molineus felineus* (from *Felis yaguarundi* from South America) and *Microstrongylus genettæ* (from *Genetta senegalensis* from Gambia, West Africa). All three have certain characters in common, viz., small size, cephalic swelling, posteriorly placed vulva and " spiked " tail in the female, and short externo-dorsal ray in the male. Each differs from the others in several important points of morphology, in addition

to the different geographical distribution. In *Molineus* the cervical ring and the excretory groove are absent ; the spicules are short with sharp points ; the ovejectors are equal. In *Microstrongylus* both cervical ring and excretory groove are present, while the spicules are long and filiform ; the ovejectors are unequal. In the present species both cervical ring and excretory groove are present but the spicules are short with sharp points. Unlike the others, prebursal papillæ are present. The ovejectors are equal. Accordingly the new generic name *Nematostrongylus* is proposed for this form with the new species *N. planicipitis* as type.

Trichuridæ.

Capillaria sp.

A single female Capillaria was found in the small intestine. The length was 5 mm. and the breadth 0·04 mm. The ova mature measure about 50μ by 20μ and are unsegmented. The female tail is rounded without any papillæ.

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Observations on the Morphology of Syngamus of some Wild and Domestic Birds.

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Introduction.

It has been shown that turkeys (Ransom, 1921, and Ortlepp, 1923) and starlings (Lewis, 1925 and 1926) play an important rôle in the distribution of gapes disease.

Schlotthauber (1860) described the gapeworm from starlings as a species distinct from that of the domestic chicken, and named it *Syngamus pugionatus* ; but, as Chapin (1925) and Baylis (1926) have pointed out, this is a *nomen nudum*.

Experiments carried out, under carefully controlled conditions, at the Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine (Leiper, 1926) showed that when infective eggs derived from the gapeworm of starling were fed to young domestic chickens gapes was produced, and heavy infections resulted. Ortlepp (1923) proved that the infective eggs from the gapeworm of the turkey also produced the disease in young domestic chickens. It seems, therefore, that the gapeworm from the turkey, and from the starling, are either of the same species as that found in the domestic chicken, or that they are of a different species or variety but can produce the same disease when present in, or transferred to, the domestic chicken.

When examining the gapeworms from the starling it was observed that the majority of the characters corresponded with those of the common gapeworm of the domestic chicken ; but there were some

differences which were not considered, by the present writer, to be of sufficient value to allow the formation of a separate species. These differences were not constant throughout, and varied even among the specimens collected from the same individual starling. It was realised, therefore, that an examination of a few gapeworms was not sufficient to determine the specificity of these parasitic worms ; and it was decided to collect further specimens from as many different kinds of domestic and wild birds as possible in order to study more carefully the morphology, and to ascertain the specific value of the various characters.

A large number of gapeworms were collected from domestic chickens turkeys and pheasants from Cardiganshire, Carmarthenshire and Pembrokeshire ; from bantams from Cardiganshire ; and from starlings and rooks from Cardiganshire and Merionethshire. Three gapeworms were collected from two blackbirds killed at Aberystwyth, Cardiganshire.

The specimens which had no fully developed eggs in the uteri are considered, in this contribution, as immature, while all others are considered as mature.

Nomenclature.

In previous publications (1925 and 1926) the present writer has referred to the gapeworm of birds as *Syngamus trachealis*. According to the Rules of Zoological Nomenclature, however, it appears that priority must be given to the name *Syngamus trachea*.

It was Von Siebold (1836) who first recognised this gapeworm as a Nematode and named it *Syngamus trachealis ;* but Montagu (1811) undoubtedly dealing with the same parasite named it *Fasciola trachea*. Von Siebold (1836) created the new genus *Syngamus*, which replaced the erroneous term *Fasciola*. Therefore *Syngamus trachea* is here recognised as the correct name.

Morphology.

Syngamus from domestic chicken.

The mature female gapeworms from chicken varied from 6·5 mm. to 28 mm. long, averaging about 13·6 mm. in length. Ortlepp (1923) however observed specimens over 30 mm. long. The internal diameter of the buccal capsule was 0·254 mm. to 0·699 mm., the external diameter 0·292 mm. to 0·825 mm., and the depth 0·195 mm. to 0·437 mm. ;

the œsophagus measured 0·582 mm. to 1·311 mm. long; and the vulva was 2·087 mm. to 4·00 mm. from the anterior end. The maximum width of the female was 0·360 mm. to 0·787 mm.; and the tail was 0·233 mm. to 0·398 mm. long.

Immature specimens of *Syngamus* from young chickens showed that the opening of the buccal capsule was directed to the anterior, but a gradual change of the axis occurred as the worm developed to maturity, Thus the mouth became directed to the dorsal in the more advanced immature, and in the mature or gravid individuals.

The capsule of the male was usually, though not invariably, in advance of that of the female. There was a well-marked annular thickening of the anterior external border of the mouth capsule characteristic of *Syngamus trachea* of chicken. The buccal cavity of the female appeared cup or bowl shaped, but in the male it often presented a wedge-shaped appearance, being wider in front and very much narrower behind. The male measured from 3·5 mm. to 6 mm. long and the internal diameter of the buccal capsule 0·215 mm. to 0·388 mm., the external diameter 0·263 mm. to 0·582 mm. and the depth 0·163 mm. to 0·407 mm.; the œsophagus 0·504 mm. to 0·661 mm. long, and the maximum width of the male 0·321 mm. to 0·410 mm.

At the base of the buccal capsule there were from six to ten buccal teeth. This variation in the number of teeth is of considerable importance as one of the characters of the genus *Syngamus* is the presence of " eight or nine teeth of two distinct sizes " (Chapin, 1925). The teeth as seen by the present writer were often of various sizes and shape (fig. 2; u, v, and w) and did not always comprise merely two distinct sizes.

Probably due to the distended uteri, the postvulvar region was thicker than the prevulvar region; the uterine complex was usually entirely posterior to the vulva and extended posteriorly to a distance a little anterior to the anus. The posterior end of the female presented a great deal of variation in shape. Hitherto it has been accepted that the posterior end of the female of the common Syngamus of chicken tapers rapidly behind the anus to a short pointed tail. A number of camera-lucida drawings (fig. 1; a, b, c, d, i, j and k) show that a series of variations may occur with a gradation from a pointed tail formed by a gradual tapering of the posterior end, to one formed by a more rapid tapering

of the posterior end and to a tail tapering gradually to end in a blunt posterior tip.

The eggs of the female were 0·075 mm. to 0·100 mm. long by 0·043 mm. to 0·046 mm. wide.

Important variations occur in the male. Up to the present the length of the spicules have been given as varying from 0·057 mm. to 0·069 mm. The spicules of 40 specimens of Syngamus from chickens were examined and measured, and it was found that they were equal, or sub-equal, and varied from 0·053 mm. to 0·082 mm. long.

The dorsal ray of the caudal bursa presents a very interesting range of variations. It may or may not bifurcate. When it does bifurcate the cleft between the two branches may be shallow or deep, and each branch may be irregularly bidigitate or tridigitate, or again one branch may be bidigitate and the other tridigitate. Asymmetrical division of the dorsal ray is often encountered. In fig. 2; a to f, a series of variations is shown which, like the rays of *Monodontus trigonocephalus* (Cameron, 1923) may be associated with recent evolution. The present writer could not find any correlation between any of the variations in the spicules and any form, or group of forms, of the divisions of the dorsal ray.

Syngamus of Turkey.

Mature female gapeworms from turkey chickens range from 13 mm. to 31 mm. long. Greater lengths than this have been obtained from turkeys, for Ransom (1921) states that "gapeworms in turkeys have have been found commonly to measure 30 mm. to 40 mm. in length, and have been found as long as 50 mm."

The buccal capsule has the annular thickening at the anterior border, as is found in the Syngamus of chicken. The internal diameter of the buccal capsule of the female ranges from 0·322 mm. to 0·723 mm., the external diameter 0·443 mm. to 0·977 mm., and the depth 0·244 mm. to 0·623 mm.; the œsophagus is 0·822 mm. to 1·277 mm. long, the maximum width of the female is 0·500 mm. to 1·000 mm., and the tail is 0·278 mm. to 0·388 mm. long. The tail generally tapers fairly rapidly to a point; the tapering varies as shown in fig 1; r to v, and in the majority of specimens examined the posterior end of the tail was bent dorsalwards.

The male ranges from 4·5 mm. to 6 mm. long ; the internal diameter of the buccal capsule is 0·278 mm. to 0·466 mm., the external diameter 0·389 mm. to 0·667 mm., and the depth 0·234 mm. to 0·322 mm.

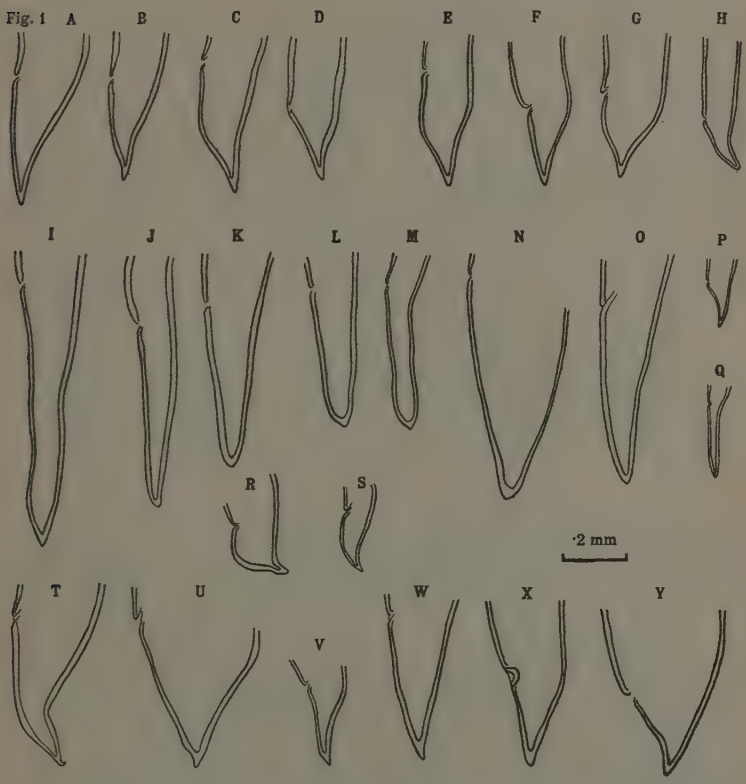

Fig. 1.—Tails of female Syngamus, from Chicken, a, b, c, d ; Pheasant, e, f, g, h ; Chicken, i, j, k ; Starling, l, m, n, o ; Bantam, p, q ; Turkey, r, s, t, u, v ; Blackbird, w, x, y.

the œsophagus is 0·612 mm. to 0·789 mm. long and the maximum width of the male 0·400 mm. to 0·514 mm.

The variations in the dorsal ray of the caudal bursa (fig 2 ; o, p and q), the length of the spicules, the number and nature of the buccal teeth are much the same as those found in the gapeworm of chicken, with the exception that the tridigitate form of the branches of the dorsal ray is more common in the turkey gapeworm, and no case was examined where ten buccal teeth were observed.

Syngamus of Bantam.

A large number of immature gapeworms were collected from bantam chickens. No mature specimens were obtainable. The longest female was 6·5 mm. and the male 3·5 mm. The annular thickening of the anterior border of the buccal capsule is well developed in these gapeworms ; the buccal teeth are much the same as in the chicken and turkey gapeworms. Variations in the form of the female tail again occur in these young gapeworms for, as shown in fig. 1 ; p and q, drawn by camera-lucida, the tail may taper rapidly to a fine point, or it may become much narrower at the level of the anus and taper gradually to form a blunt posterior end. The spicules vary from 0·063 mm. to 0·070 mm. The dorsal ray of the caudal bursa is normally tridigitate, but variations corresponding to those of the dorsal ray of the chicken gapeworm occur. In one case one of the branches of the dorsal ray showed no digitations.

Syngamus of Pheasant.

The mature female gapeworm from young pheasants ranges from 8 mm. to 22 mm. long, the maximum width being 0·324 mm. to 0·682 mm. The internal diameter of the buccal capsule is 0·185 mm. to 0·477 mm., the external diameter 0·277 mm. to 0·625 mm. and the depth 0·203 mm. to 0·454 mm. The œsophagus is 0·703 mm. to 1·125 mm. long ; and the vulva is 2·5 mm. to 5 mm. from the anterior end. The annular thickening of the external border of the buccal mouth is similar to that of *Syngamus trachea* of chicken. The buccal teeth vary in shape and may be from eight to ten in number. The tail of the female (fig. 1 ; e to h) tapers rapidly to a point posteriorly to the anus. The tapering, however, may commence at the level of, or well behind, the anus. No blunt tail was observed.

The male varies from 4 mm. to 5·75 mm. long ; the internal diameter of the buccal capsule is 0·145 mm. to 0·250 mm., the external diameter

0·184 mm. to 0·277 mm., and the depth 0·126 mm. to 0·185 mm. The œsophagus is 0·509 mm. to 0·658 mm., and the maximum width is 0·194 mm. to 0·317 mm.

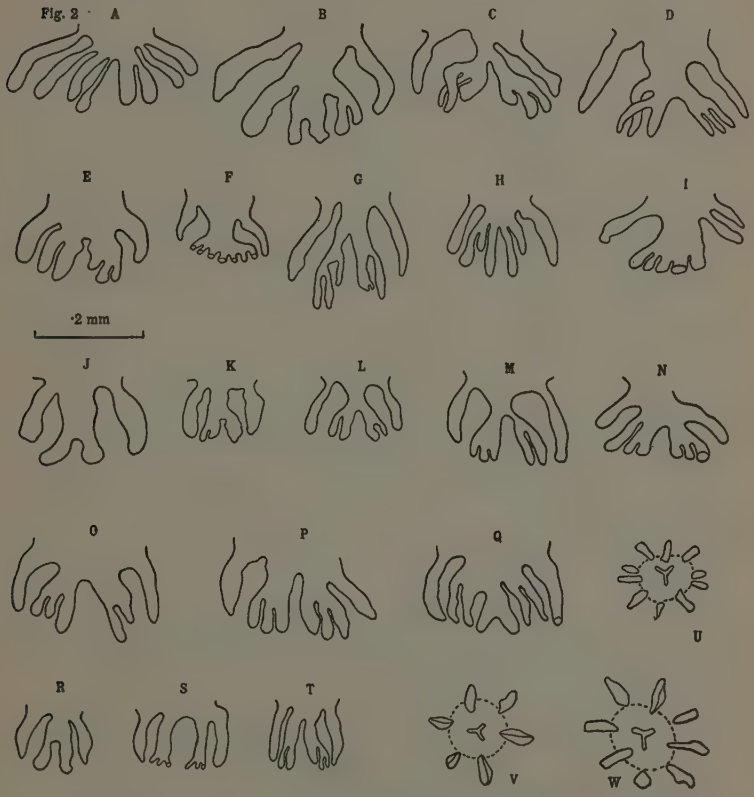

Fig. 2.—Tails of male Syngamus from Chicken, a, b, c, d, e, f; Pheasant, g, h, i; Starling, j, k, l, m, n; Turkey, o, p, q; Blackbird, r, s; Rook, t. Buccal teeth of Syngamus from Starling, u, v, w.

The spicules are equal or sub-equal and 0·059 mm. to 0·069 mm. long. The dorsal ray of the bursa presents a number of variations (fig. 2; g, h and i) resembling some of those found in *Syngamus trachea* of chicken.

It may divide into two similar branches, each of which may again divide to form three equal or unequal digits, or it may form two unequal branches each with a varying number of digitations.

In all other respects the gapeworm from pheasant resembles *Syngamus trachea* from chickens.

Syngamus of Blackbird.

Only three specimens, all mature, were obtained from blackbirds examined. The female gapeworm varied from 8 mm. to 10 mm. long. As stated by Baylis (1926) there is no annular thickening to the external anterior border of the buccal capsule. The internal diameter of the buccal capsule (of the gapeworm 10 mm. long) is 0·203 mm., the external diameter 0·320 mm., the depth 0·242 mm. ; the œsophagus 0·805 mm. long, and the vulva 2·338 mm. from the anterior end. The maximum width of the female 0·485 mm. The tail measures 0·427 mm. long.

The tails of the three specimens are shown in fig. 1 ; w, x and y. It may be seen that the tapering begins near the anus and ends in a pointed tail which is, however, not so finely pointed as that figured by Baylis.

A male specimen 2·5 mm. long has an internal diameter of the buccal capsule of 0·116 mm., the external diameter 0·194 mm. and the depth 0·174 mm. ; the œsophagus is 0·572 mm. long and the maximum width 0·271 mm.

The spicules seen were unequal, the longer ones being 0·064 mm. to 0·078 mm. long. The dorsal ray (fig. 2 ; r and s) varies in this case also. In one specimen the ray divided into two simple branches by a deep cleft ; in the other the two branches are separated along the whole length. Each branch possessing three digitations which can only be made out clearly under high power (1/6″).

In all other respects these gapeworms resembled *Syngamus trachea*.

Syngamus of Starling.

In a previous publication (Lewis, 1926) the range of variation in the length of the gapeworm of starling was given as 11 mm. to 18 mm., and the average length as 13·6 mm. Further specimens were collected and measured with the result that the range of variation in the length of mature gapeworms from starling must be stated as 7 mm. to 18·5 mm., the average length being about 13 mm. Only one immature specimen was obtained—it was 6·3 mm. long. Gapeworms of 7 mm. long

contained but a few eggs in the uteri. The relation of the buccal capsules of paired specimens is very much the same as that found in *Syngamus trachea* of chicken. The annular thickening of the anterior margin of the buccal capsule is usually like that of *Syngamus trachea*, but occasionally it is wider, which causes the appearance of a very much larger capsule. The buccal capsule dimensions are as follows :—the internal diameter 0·207 mm. to 0·825 mm., the external diameter 0·256 mm. to 1·000 mm., and the depth 0·194 mm. to 0·394 mm. The œsophagus varies from 0·439 mm. to 1·116 mm. ; the vulva is 1·068 mm. to 3·912 mm., from the anterior end. The maximum width is 0·388 mm. to 0·679 mm.

The male measures 2·5 mm. to 4·5 mm. long. The internal diameter of its buccal capsule is from 0·194 mm. to 0·242 mm., the external diameter 0·291 mm. to 0·340 mm., and the depth 0·126 mm. to 0·242 mm. The œsophagus is 0·380 mm. to 0·601 mm. long, and the maximum thickness is 0·291 mm. to 0·339 mm.

The number of teeth projecting from the base of the buccal capsule varies from six to nine, and are not of a constant, unvarying shape. No specimen with seven or ten teeth was observed. The dimensions of the eggs are as those of *Syngamus trachea*. The uterine complex may occasionally project slightly anterior to the vulva, but this is not usual. Posteriorly the uterine complex extends to a distance a little anterior to the anus.

The tail of the female is 0·291 mm. to 0·539 mm. long, and tapers gradually to a blunt point (fig. 1, l to o). But, whereas the tail varies in the degree of bluntness, no specimen was observed where the tail tapered to a fine point.

The spicules of the male are equal or subequal and range from 0·054 mm. to 0·074 mm. long. The dorsal ray presents a range of variations somewhat different from the *Syngamus trachea* of chicken. In one specimen each branch of the bifurcation was simple, and a gradation as shown in fig. 2 ; j to n, was observed. The tridigitate form of each branch was common, though the digitation was in no case absolutely symmetrical. It may be noted that the variation of the dorsal ray seems to advance from the simple bifurcation to the bidigitate and tridigitate bifurcations, whereas in the *Syngamus trachea* of chicken the variations observed by

the writer show an advance from the digitate and tridigitate forms to a more complicated and irregular digitation.

Syngamus of Rook.

No immature gapeworms were obtained from the rook. The length of the mature female ranges from 4 mm. to 12 mm. The maximum width is 0·339 mm. to 0·582 mm. The annular thickening external to the anterior border of the buccal capsule corresponds with that of *Syngamus trachea*. The internal diameter of the buccal capsule varies from 0·271 mm. to 0·514 mm., the external diameter 0·339 mm. to 0·631 mm., and the depth 0·184 mm. to 0·339 mm. The œsophagus is 0·679 mm. to 0·873 mm. long, and the vulva 0·601 mm. to 0·805 mm. from the anterior end. The buccal teeth vary from eight to nine in number. The uterine complex is similar to that of *Syngamus trachea* and the eggs are within the range of variation found in that species. The tail is 0·351 mm. to 0·534 mm. long and tapers to a blunt end posteriorly.

The male ranges from 1·5 mm. to 3·6 mm. long and 0·194 mm. to 0·275 mm. wide. The internal diameter 0·194 mm. to 0·275 mm., and the depth 0·145 mm. to 0·214 mm. The œsophagus is 0·485 mm. to 0·592 mm. long. The spicules are equal or subequal and vary from 0·065 mm. to 0·073 mm. long. Of the two caudal bursæ successfully dissected one showed an irregular tridigitate form of the two branches of the dorsal ray; the other showed one branch bidigitate and one tridigitate (fig. 2; t).

Discussion.

Chapin (1925) separated the genus *Syngamus* v Siebold, 1836 from *Cyathostoma* E. Blanchard, 1849, on the following grounds: In *Syngamus* the sexes are permanently joined in copula; there are eight or nine teeth of two distinct sizes at the base of the buccal capsule, the bursal rays are short and thick, and the spicules small to very small (150μ to 25μ); in the genus *Cyathostoma* the sexes are not permanently joined in copula, the buccal teeth are six to seven in number and of two distinct sizes, the bursal rays are slender, and the spicules long (more than 400μ) and filiform.

A careful examination of a large number of specimens from various birds shows that the teeth projecting from the base of the buccal capsule

are not always of merely two distinct sizes; they vary much in size and in shape. The number of teeth is not restricted to eight or nine; they may be six, eight, nine or even ten (fig. 2, u, v and w). Therefore the number of buccal teeth does not serve as a character distinguishing these two genera, unless it may be said that in *Cyathostoma* the teeth do not exceed seven in number.

Syngamus trachea, the common gapeworm of chicken, and type species of the genus *Syngamus*, has hitherto been recognised by the following characters. The sexes are joined in permanent copula, the mouth of the buccal capsule is directed dorsally in mature specimens, the buccal capsule is provided with an annular thickening exterior to its anterior border; there are eight or nine teeth arising from the base of the buccal capsule; the uterine complex is posterior to the vulva and extends to a distance a little anterior to the anus; posterior to the anus the body of the female tapers rapidly to form a short pointed tail; eggs are 0·078 mm. to 0·100 mm. long and 0·043 mm. to 0·046 mm. broad: spicules of the male 0·057 mm. to 0·069 mm. long; the dorsal ray of the caudal bursa bifurcated for about half its length, or more, each branch being bi- or tri-digitate; the externo-dorsal ray simple, fairly short and thick, the three lateral rays are parallel, close together and roughly equal in thickness; the two ventral rays similar.

From the description of the morphology of *Syngamus* from chicken given in this paper it must be concluded that the parasite is *S. trachea*, but the specific characters are subject to much variation and elasticity. Even in two worms of the same length, and from the same host the characters vary very greatly. It is shown that *S. trachea* from the chicken varies a great deal in length, in the form and size of the buccal capsule, in the nature and number of buccal teeth, in the form of the female tail, in the dimensions of eggs, in the division of the dorsal ray of the caudal bursa; and there is occasionally a slight variation in the thickness of the lateral rays. But these variations merge one into the other and do not seem to allow the formation of different species. They suggest, however, that species of the genus *Syngamus* are much influenced by the conditions of the environment in which they live and that these variations arise as a result of their adaptability and elasticity.

In view of the relatively wide range of environmental conditions to which they are subject as inhabitants of the trachea, members of the

genus *Syngamus* may be considered as peculiarly liable to modification. It may be suggested that the necessity of remaining fixed may lead to individual variations in the growth of the rays in relation to the particular plane of attachment on the vulvar protuberance. It is also reasonable to expect that the modifications which *S. trachea* may undergo when transferred from a bird of one kind to a bird of another kind may be greater than if it were transferred to another bird of the same kind ; and indeed, such modifications may be so great that the gapeworm may assume characters apparently sufficient to justify the formation of a new species. But if a large number of specimens are examined it is seen that there is a gradation of variations which suggest that this species is the same, but influenced, to a degree, by the different environment.

The length of the gapeworm, at maturity, varies in the birds examined. In the chicken, turkey, pheasant, starling and young rook the lengths are 6 mm., 12 mm., 8·75 mm., 6·3 mm., and 4 mm. respectively. This may probably be correlated with the habitat of the parasite.

Each branch of the dorsal ray of the caudal bursa of the male *Syngamus* of the starling is commonly irregularly tridigitate but the gradation of variation commences with two simple branches of the bifurcation, then advances to the case where one branch is simple while the other is bidigitate and on to where both branches are bidigitate, and then to the tridigitate form. In the *Syngamus* of chicken a series of gradations occur from a bidigitate form to a form where the dorsal ray does not bifurcate but possesses seven finger-like terminations ; or it divides into six similar branches which may correspond to the six digitations of the two normal branches.

In the case of the chicken gapeworm the tridigitate branches are common. Such also is the case in the gapeworm of the turkey, the pheasant, and the bantam. In the gapeworm of the rook the bidigitate and tridigitate form of the dorsal ray is present ; and in the gapeworm of the blackbird the simple bifurcate or the tridigitate form of the dorsal ray may occur (fig. 2, r. and s).

It would, therefore, seem inadvisable to allow much weight to differences in the dorsal ray in any attempt to discriminate species of *Syngamus*.

The posterior end, or tail, of the female presents a series of variations

from a fine pointed tail to a very blunt one. In the gapeworm of the chicken the tail clearly presents this series. In the turkey and pheasant the tail of the gapeworm is not blunt but tapers to a point which may be relatively acute or relatively obtuse. In the bantam the tail may be fine and pointed or blunt, but in the gapeworm of the starling and the rook the tail is always blunt. Baylis (1926) describes and illustrates the tail of *Syngamus merula* from *Turdus merula* as tapering gradually to a fine point. Gapeworms collected from *Turdus merula* by the present writer possessed tails tapering rapidly to an obtuse point. Chapin (1925) notes that the tails of *S. parvus* and *S. gracilis* are blunt. As far as this character of the tail is concerned all the gapeworms referred to above may be considered as variations of *S. trachea*. *S. parvus*, however, may be distinguished from the latter by the length of the spicules and the nature of the lateral rays; and *S. merula* by the consistent absence of the annular thickening external to the anterior border of the buccal capsule. It would be more satisfactory, however, to examine further specimens of these two species and of *S. gracilis*, in order to ascertain the constancy of the distinguishing characters, and to establish more firmly their specific value.

The writer considers that all the gapeworms—apart from that collected from the blackbird—examined by him are of the species *Syngamus trachea*.

Chapin (1925) states "it is further probable that *Syngamus trachea* which can hardly sustain itself in a host as close phylogenetically to the turkey as the domestic fowl is, cannot under even the most favourable circumstances reach maturity and egg production in the crow. From this it seems probable that the crow is not, as often stated, a reservoir for the turkey and chicken gapeworm." It seems quite clear, however, that the gapeworm from the rook—a corvine host—is *Syngamus trachea*. Furthermore, the turkey and pheasant are more delicate birds than the domestic fowl and may on this account find it more difficult to rid themselves of the gapeworm and so act as more efficient carriers than the domestic fowl which more often succeeds in expelling the parasite. The length of the trachea may perhaps also favour the persistence of the gapeworm in turkeys and pheasants. The pheasant, starling, rook jay, blackbird and thrush are more active than the domestic fowl, and are more often subjected to adverse conditions which may so affect

the constitution of these birds that the gapeworm is enabled to sustain itself in the windpipe of its host.

It is, however, desirable to carry out further experiments by feeding chickens with the infective eggs from gapeworms of various wild birds, and to study the influence of the changes of environment on the parasite.

ACKNOWLEDGMENTS.

I am indebted to Professor R. Douglas Laurie for helpful advice during the time this work was carried out.

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On a New Species of the Nematode Genus *Oswaldocruzia* from the Newt.

By E. Aneurin Lewis, M.Sc.

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Introduction.

The following description of a new species of the genus *Oswaldocruzia* is based on the examination of a considerable number of specimens collected from the intestines of 80 newts caught at Aberystwyth. They were found to be equally common in the Smooth Newt (*Molge vulgaris* Linn.) and the Palmate Newt (*Molge palmata* Dum. and Bibr.).

Oswaldocruzia molgeta sp. nov.

The worms are white in colour and taper towards the anterior and the posterior ends. The mouth is surrounded by three small indistinct lips, each of which bears a pair of very small papillæ. The cephalic extremity has the cuticle dilated, the dilation being divided into a wider anterior portion and a narrower, slightly longer, posterior portion. The former is marked off from the latter by a fine constriction. Faintly marked transverse striations may be seen on the cuticle of the dilations, but they can only be made out by examination with the oil-immersion lens.

The œsophagus is long and claviform. The nerve ring is situated at about half-way down the length of the œsophagus, and a little anterior to the excretory pore. Mid-way between the nerve ring and the posterior end of the œsophagus are situated two small cervical papillæ.

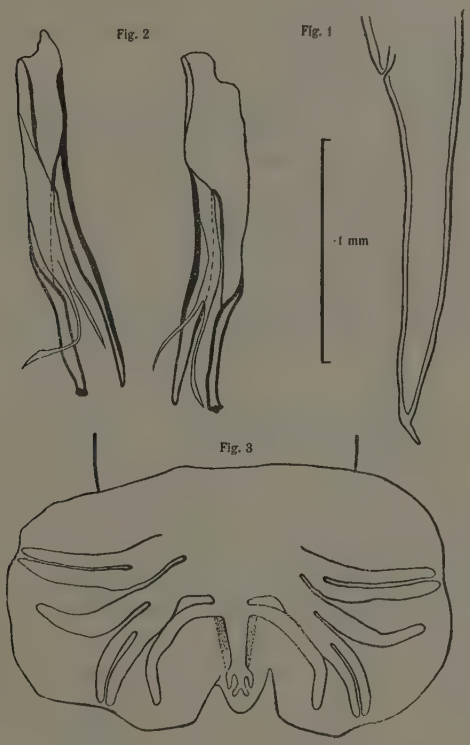
The *female* varies from 5 mm. to 8·5 mm. long with an average length

of 7 mm.; the maximum breadth in the region of the vulva varies from 0·131 mm. to 0·159 mm. The width across the anterior cephalic dilation is from 0·032 mm. to 0·043 mm., and across the posterior cephalic dilation from 0·030 mm. to 0·035 mm. The œsophagus varies from 0·412 mm. to 0·515 mm.; the execretory pore, which is usually quite distinct in freshly fixed specimens, is situated at about 0·300 mm. from the anterior end. In some specimens it was observed that the margins of the excretory pore were raised into fairly prominent lips. The vulva is a transverse slit and is situated just within the posterior third of the body; it does not possess prominent lips as in the case of *O. filiformis*. The ovejecter is short, about 0·037 mm. long, and runs a little distance in a transverse direction to open into two divergent uteri, one branch leading to the anterior part of the body and the other to the posterior. The uteri are long and contain many eggs in various stages of development. No embryonic eggs were observed. The eggs are ellipsoidal with a thin egg-shell, and measure 0·075 mm. to 0·093 mm. in length, and 0·042 mm. to 0·047 mm. in breadth.

The posterior part of the body tapers rapidly behind the anus to form a fine pointed tail 0·15 mm. to 0·159 mm. long. The posterior extremity of the tail is marked off from the greater portion of its length by a conical, dorsally bent tip (fig.1).

The *male* is smaller and narrower than the female. It has a length ranging from 3·5 mm. to 6 mm., and a breadth of about 0·084 mm. The anterior part of the body is similar to that of the female; posteriorly the body ends in a small blunt cone, surrounded by the caudal bursa. The caudal bursa is about 0·160 mm. wide, and distinctly tri-lobed (fig. 3). The ventro-ventral and the latero-ventral rays are close together, approximately equal, and extend to the margin of the bursa. The externo-lateral is separated from the medio-lateral and posterio-lateral, which are close together and extend to the margin of the bursa. The externo-lateral is shorter than the other lateral rays, and has a definite ventral curve. The externo-dorsal arises from the base of the dorsal trunk; it is long but due to its curvature it does not reach the border of the bursa. The dorsal ray is thick and divides, within the small dorsal lobe, into a number of digitations to which Travassos (1921) gave the term " chapiteau de papillæ." Surrounding the trunk of the dorsal ray there is, invariably, a mass of precipitate the chemical nature

and the function of which seems to be unknown. The digitations of the "capital" of the dorsal ray may vary considerably.



Oswaldocruzia molgeta sp. nov.
Fig. 1. Tail of female. Fig. 2. Spicules.
Fig. 3. Bursa of male.

The spicules are equal in size and strongly chitinised, and vary from 0·157 mm. to 0·187 mm. long. They are divided into three main branches, the central branch being the least chitinised, and again divided into two, the total number of processes being four. The three branches vary in form as shown in the illustration (fig. 2).

Discussion.

The structure of the head, and the striated dilation of the cephalic cuticle, the formation of the bursal rays with the dorsal ray like a capital of a column, the multiple processes of the spicules, and the absence of a gubernaculum, justifies the allocation of this species to the genus *Oswaldocruzia* Travassos, 1917.

In his monograph on the Trichostrongylidæ (1921), Travassos gives all the available descriptions of seven species of Oswaldocruzia. At the end of these descriptions he adds the following : " Les 7 espèces de ce genre semblent pouvoir être réduite à 2, parce que les autres sont ou des synonymes ou alors n'appartiennent à ce genre." According to Travassos the only two valid species hitherto recorded, and fully described, are *Oswaldocruzia subauricularis* and *O. filiformis*.

The length of the worms of the species described above, the length of the ovejecter, the form of the female tail, the dimensions of the eggs, the length of the spicules, and the number of spicular processes, differentiate this species from the other two. Therefore I propose the name *Oswaldocruzia molgeta* for the species here described.

It is worthy of attention, however, that in *O. molgeta* there are two cervical papillæ which are not mentioned as present in *O. subauricularis* and *O. filiformis*. Possibly these have been overlooked as they are so small. In any case the presence of cervical papillæ in *O. molgeta* does not seem sufficiently important to debar it from this genus, particularly in view of the possibility that they have been missed in the previous species.

Specimens of *Oswaldocruzia molgeta* will be deposited at the Institute of Agricultural Parasitology, London School of Hygiene and Tropical Medicine.

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Journal of Helminthology, Vol. VI, *No.* 2, *June*, 1928, *pp.* 117-120.

The Occurrence in Cyprus of *Bullinus contortus* in the Endemic Area of *Schistosoma hæmatobium*.

By R. T. Leiper, M.D., D.Sc., F.R.S.

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The occurrence of Bilharzia infection in Cyprus was first recorded by Dr. George A. Williamson in a paper entitled "*Bilharzia hæmatobia* in Cyprus," published in the *British Medical Journal* of 27th September, 1902.

Five years later the same author described fifteen cases including eight among the children in the local school at Syrianokhori, a small village on Morphou Bay on the west coast of the Island.

Sir Ronald Ross, in his Report to the Colonial Office on the Prevention of Malaria in Cyprus (1913), drew attention to a village Syrianokhori, where "the few children found in the school gave a spleen rate of 100 per cent." He remarks that the village "also suffers from Bilharzia disease, due to the river," and in his "Miscellaneous Suggestions" he adds "efforts should also be made to prevent Bilharzia disease in this village as, if care is not taken, that very serious disease is likely to spread in the Island." At that time of course the rôle of molluscs, in the spread of Bilharzia disease, had not been determined.

As no one appeared to have looked into the question since that time, I took the opportunity of making a brief visit to Cyprus on my return journey from Egypt to England in February, 1928. His Excellency, Sir Ronald Storrs, C.M.G., C.B.E., who had at one time been attached to the Residency in Cairo and was well acquainted with the subject, gave me every facility to pursue local enquiries. I was accompanied by the Medical Officer of Health, Dr. A. S. Millard, and the Chief Sanitary Inspector, Mr. Aziz, to Syrianokhori, and for whose helpful co-operation I am much indebted.

On the books of the village school there were seventeen boys, four of whom at the time of my visit were found to have eggs of *Schistosoma hæmatobium* in the urine. A visit was made to the river which at that time of the year was a torrential stream. No molluscs were found along its banks. The district in which the village is situated is irrigated by a series of deep artificial channels bringing the water from the river at some distance upstream. During a search extending over two days, a few specimens of *Bullinus* were found. During the month of February it was evidently an uncommon species, but the irrigation channels were heavily stocked by a species of *Melanopsis* which I had not observed in Egypt. The drains below the village were full of weed in which were numerous Anopheline larvæ and considerable numbers of *Limnæa.*

According to local medical reports, Syrianokhori is the only village in the Island infected with Bilharzia. Few cases are known in Morphou about a mile inland from Syrianokhori and one school boy there was found who had the disease, but these cases attribute their infection to visits to Syrianokhori for purposes of fishing or bathing. It is stated that during the summer months the flow of the river ceases and it is from bathing and fishing in pools left in the bed of the river that infection is acquired.

The Chief Veterinary Surgeon, Mr. R. J. Roe, M.R.C.V.S., who kindly gave me facilities for microscopical work in his laboratory during my stay in Cyprus, motored me to various other parts of the Island for purposes of collecting. In no other part did I find specimens of *Bullinus* although *Limnæa spp.* and *Melanopsis* sp. were common.

It is difficult to explain the origin of *Schistosoma hæmatobium* at Syrianokhori. Although no infected *Bullinus* were found at the time of my visit it seems very improbable that any of the other mollusca found are the carriers. The month of February is the coldest month of the year in Cyprus. In Egypt no infected *Bullinus* occurred at the same time of the year.

According to the geologists Cyprus was at one time connected by land with the mainland of Asia Minor from the north-east towards the Gulf of Iskanderun. We have very little knowledge, however, either of the molluscan fauna or of the actual occurrence of *S. hæmatobium* of this part of the Asiatic mainland. As the name of the village indicates, the inhabitants are the descendants of Syrian

immigrants. It is doubtful, however, if they brought with them either the disease or the molluscan carrier from the mainland.

Bullinus contortus has now been reported from several of the Mediterranean Islands and it is probable that at an early period, it was widespread throughout the Mediterranean basin before this became submerged by the waters of the Atlantic.

The diseases possibly entered the Island at a much later date and may be an additional indication of Egyptian invasions of which there are archæological traces in the neighbourhood of Syrianokhori. Indeed it is even possible, in view of the remarkably circumscribed endemic area, to suppose that the galleys which brought the early Egyptian invaders may have conveyed also specimens of intermediate hosts in their fresh water supplies.

Since the above was set in type the following communication has been received from Dr. G. A. Williamson, whose work has been referred to therein :—

> " I was greatly interested in reading the account in the *British Medical Journal* of the meeting of the Section of Tropical Medicine and Parasitology of the Royal Society of Medicine on 3rd May to find that you discovered Bilharzia Disease still in the same endemic focus (Syrianokhori, near Morphou) as I found it some twenty years ago ; at that time the connexion with Fresh Water Snails was not known and like everyone else I imagined (though it seemed very strange) that the miracidium had some means of entering the body through the skin ; during the war I happened to be in Cyprus and had an opportunity to pass through Syrianokhori where I searched for snails, and found what appeared to me to be specimens of *Bullinus*, but had no means of having this point corroborated—no one there knew anything about them ; from comparison with definitely recognised Bullinus shells I am convinced I was correct in my diagnosis.
>
> You found four out of seventeen boys examined had ova in their urine—I, in 1907, found eight boys in the village school, one was unable to pass water at the moment but out of the remaining seven no less than five had ova.
>
> The name Syrianokhori (" Syrian village ") is very suggestive as to the origin of the disease.

In view of the fact that the focus is so very restricted I hope an effort will be made by the Government to deal with the situation —I feel it could be effectually done."

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The Species of the genus *Aphelenchus*.

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Introduction.

In a paper published towards the end of last year the author (Goodey, 1927) described a new free-living species of Aphelenchus, *A. winchesi*, and dealt with the type species of the genus *A. avenæ*, discussing its affinities to certain nearly related species. A further paper was promised dealing with the various species of the genus and the present communication is an attempt to fulfil that promise.

Plant Parasitic Species.

Before dealing with these seriatim, a few general considerations on the validity of the species associated with diseases in plants may be stated. In this connection it is interesting to compare them with the case of *Tylenchus dipsaci* which is regarded as capable of attacking a large range of plants and of becoming physiologically adapted to a particular host to the extent of establishing a definite biologic strain peculiar to that host. Such strains are morphologically indistinguishable from one another.

In the case of some of the plant-parasitic Aphelenchi, on the other hand, the same concept as to specificity cannot be said to apply. Each disease is considered to have its own particular species of Aphelenchus

as causative agent and yet these differ from each other only in dimensions and not in morphological details, *e.g.*, the worms found in strawberry, chrysanthemum and in black currant bud disease.

That the present situation in this matter is unsatisfactory is probably recognised by all who deal with these organisms. It is exemplified in a recent note by Cobb and Steiner (1927) communicated to the Helminthological Society of Washington which concludes as follows —" What is needed is a careful re-examination and comparison of Aphelenchi from various host plants and experiments on the reaction of these plants to the same form. Such researches may prove *A. fragariæ* Ritzema-Bos, *A. olesistus* Ritzema-Bos, *A. ritzema-bosi* Schwartz, and *A. subtenuis* Cobb to be a single species. If so, it is important to know it."

Earlier workers carried out investigations in this direction. Marcinowski (1908) put forward the view that *A. fragariæ*, *A. olesistus* and *A. ormerodis* should be considered as identical forms and she selected the specific name *A. ormerodis* as the most suitable because, as the worm was probably polyphagous in habit, it was preferable to choose a name for it which did not indicate a particular host relationship. She conducted some experiments in which attempts were made to infect begonia and strawberry plants with Aphelenchi from diseased orchids. She succeeded in setting up typical disease symptoms in the begonias, already listed as a host for *A. olesistus*, and a certain amount of leaf-blotch in the strawberry plants but not hypertrophy of the stem tissues.

Her experiments must be regarded as inconclusive on the question of whether one and the same species of Aphelenchus can cause disease in orchids and strawberries.

Taylor (1917) also attempted to set up infection of strawberry plants with Aphelenchi from diseased black-currant buds by growing them right under infected black currant bushes, but in no case were symptoms of disease observed on the parent plants or on their runners though the experiment was continued for two seasons.

Both Marcinowski's and Taylor's experimental results leave the main problem untouched and still open for investigation. It seems clear that the relationship of parasite to host is a very specialised one

and that it is extremely difficult to get Aphelenchi parasitic on one host to attack another plant known to be capable of harbouring parasitic Aphelenchi.

Since the writer's earlier paper reviewing the plant parasitic Aphelenchi (Goodey, 1923) opportunities have occurred from time to time for making detailed observations on members of the genus occurring in ferns, strawberry, black currant and chrysanthemum. As a result of these it has not been possible to distinguish any anatomical differences between the forms found in strawberry, black currant and chrysanthemum. The male spicules have the same shape and the male caudal papillæ are the same in number and have the same distribution in all three. Similarly the arrangement of the œsophageal structures and the salivary glands is the same in all. Again, the species attacking chrysanthemum differs from *A. fragariæ* only in its greater size.

A. olesistus has been obtained from several genera of ferns and does present certain differences from *A. fragariæ*, *A. ritzema-bosi* and *A. ribes* which will be discussed when the species is dealt with.

Aphelenchus fragariæ Ritzema-Bos, 1891.

This species was first described from strawberry plants suffering from "cauliflower" disease or as Cobb afterwards called it "bunch." Its connection with the so-called "red-plant" disease of strawberry has been the subject of a good deal of recent investigation, particularly at the Long Ashton Research Station, and the views of the workers there are given in a paper by Ball, Mann and Staniland (1927). They say, p. 631, that "the worms can be present on plants without causing disease symptoms" and "it must not be overlooked that the view that the organisms are the casual agents of disease is so far purely circumstantial and needs to be supported by positive results from infection experiments before it can be claimed with certainty that *Aphelenchus fragariæ* is the cause of "cauliflower" or "red plant"."

The writer has found *A. fragariæ* in deformed leaves of three strawberry plants in the garden of the Institute's field station at Winches Farm. The plants showing these leaves were in a poor condition with considerable browning and death of the outer parts of some of the larger leaves whilst towards the centre of the plants there were a few

small deformed and very twisted leaves in the tissues of which numbers of the worms were found.

Several plants growing on the same small plot were examined for the presence of worms, but only in the case of three plants were they found, although a large number of apparently similarly deformed leaves were teased up and examined microscopically.

It would be unwarrantable from this limited amount of material to claim that the worms are the cause of the deformation of the leaves, but it is clear that they can lead an endoparasitic life within the leaf substance of the deformation of which they may be the cause.

Marcinowski (1908) described and figured deformed strawberry leaves very similar in appearance to those found by the writer, and she also obtained worms from the leaf tissues.

Ritzema Bos (1891) gave the following measurements :—females, 0·57 mm. to 0·80 mm. long ; males 0·59 mm. to 0·85 mm. long with the proportion of length to breadth 46 to 50 for the females and 45 to 52 for the males. The worms found by the writer had the following measurements :—females, 0·84 mm. to 0·92 mm. long ; males, 0·74 mm. to 0·84 mm. long.

In the principal anatomical features the worms resemble the type species of the genus dealt with in the writer's recent paper (Goodey 1927) and do not call for a very detailed description here. The head end is boss-shaped and is set off from the body by a slight groove. Its sides are outwardly curved and by careful focusing on the surface, the outlines of the six lips surrounding the mouth can be distinguished. These are all fused together to form the boss. The tip of the female tail bears a small process which may have from 1 to 3 small points. At the centre of the anterior end is a small buccal opening leading inwards almost to the depth of the head ; its walls are slightly thickened. The stylet is made up of two parts, an anterior conical portion which fits onto the longer cylindrical posterior part at the base of which are the three swellings. There appear to be two stylet guides visible as small dots on either side of the stylet, each pair being connected by a very fine line.

The alimentary tract has the same structure as that of the type species. There is first a rather narrow œsophagus followed by a

prominent muscular bulb after which the second part of the œsophagus gives off the three salivary glands which lie dorso-laterally to it and the beginning of the intestine. The walls of the latter are generally fairly stout and contain numerous fatty food globules. A narrow rectum connects the intestine with the anus. The nerve ring crosses the beginning of the salivary glands and the posterior œsophagus a short distance behind the muscular bulb and the excretory pore is to be found just posterior to the nerve ring (fig. 1).

The male tail has been studied by the writer in considerable detail. In specimens fixed over the flame or by hot fixatives the tail is always curved ventrally almost into a semicircle. There are apparently three pairs of caudal papillæ ventro-laterally situated as in the free-living *Aphelenchus parietinus*, as follows: one pair ad-anal or immediately post-anal, one pair a little behind a point midway between the anus and the tip of the tail and one pair practically terminal in position just at the base of the final caudal process. The ad-anal pair has not previously been recorded for *A. fragariæ*, but the writer has found it by carefully focusing on the surface of the body under the oil-immersion. With regard to the terminal pair, it is frequently difficult to define their exact limits as in many cases there is only a slight swelling in this region and one cannot distinguish the core of the papilla so easily as in the median pair, which is the most easily seen of all.

The spicules have the thorn shape characteristic of the genus and each is made up of two dorsal pieces forming together a hollow dorsal limb and a single ventral piece. The point of the dorsal limb is really the point of the spicule, whilst anteriorly the inner part of the piece joins with the outer and a single strand from both curves over to join with the anterior end of the ventral piece. The point of the latter does not reach to the point of the dorsal limb. A hollow space occupies the centre of each spicule and the points of dorsal and ventral pieces appear to be connected by a fine strand which thus surrounds the wide orifice leading to the hollow centre.

The dorsal limb has a greater length than is usually given it. As measured by the writer it varies from 0·021 mm. to 0·023 mm. in length as compared with 0·013 mm. to 0·015 mm. given in the 1923 paper, whilst the ventral piece measures 0·01 mm. in length.

Aphelenchus ritzema-bosi Schwartz, 1911.

Syn. *A. phyllophagus* Stewart, 1921.

This species has been the subject of a good deal of observation and has been described in detail by Stewart. The writer has studied its structure very carefully during the preparation of the present paper. Anatomically it appears to be identical with *A. fragariæ* both at the head end, in the length of the stylet with its two guides, in the muscular œsophageal bulb, the arrangement of the salivary glands, the situation of the nerve-ring and excretory pore, and in the details of the male tail.

The only constant difference between the two species is that of size; *A. ritzema-bosi* is on the whole larger than *A. fragariæ*, and on this alone can they be separated.

Schwartz gave the following measurements:—females, 0·816 mm. to 1·248 mm. long; males, 0·88 mm. to 1·232 mm. long. Stewart gave females, 0·923 mm. long and males, 0·965 mm. long.

The following measurements have been obtained by the writer on worms killed over a small flame and measured immediately after death:—females, 0·87 mm. to 1·2 mm. long; males, 0·85 mm. to 0·96 mm. long.

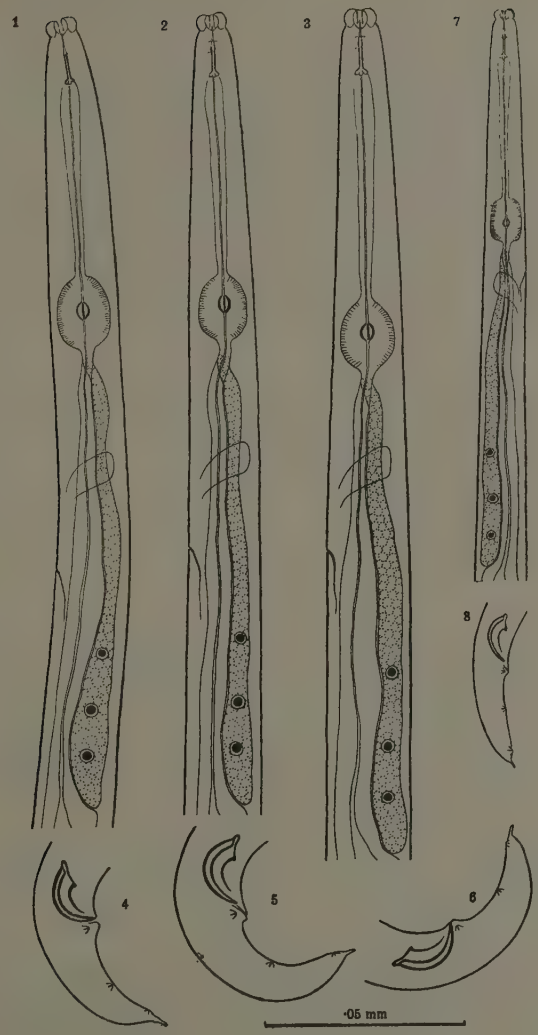
There can be no doubt, from the observations of numerous workers, that this species is capable of causing serious disease to chrysanthemums by attacking the mesophyll tissues of the leaves (*See* Schwartz 1911, Stewart 1921, Roszypal 1926, Goffart 1927).

The writer was supplied with some chrysanthemum stems bearing diseased leaves in December, 1927, by Dr. W. F. Bewley of the

Figs. 1, 2 and 3.—Anterior ends of *A. fragariæ*, *A. ritzema-bosi* and *A. ribes* respectively. Drawn from fresh specimens killed by heat. The three species are structurally indistinguishable. Note the excretory pore well behind the nerve ring.

Figs. 4, 5 and 6.—Male tail of *A. fragarise*, *A. ritzema-bosi* and *A. ribes* respectively. Drawn from fixed specimens mounted in weak glycerine. The spicule and the caudal papillæ are the same in all. Note the marked ventral curvature of the tail.

Figs. 7 and 8.—*A. olesistus*. Anterior end and male tail. Note the excretory pore in advance of the nerve ring and the slight curvature of the tail.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Cheshunt Experimental Station. By soaking the leaves in water large numbers of adult worms were obtained whose structure was studied in detail. The leaves were then placed close to the surface of two flower pots almost filled with a fresh potting compost and the pots were kept watered. About the middle of January, 1928, several cuttings of chrysanthemum, taken from a plant growing in the open border, were put into the pots with a little sand to assist them in rooting. The cuttings were very healthy in appearance and were negative for the presence of eelworms of any kind when examined microscopically. In one pot six cuttings were put and in the other four, together with two small strawberry runners taken from the open bed. Both pots were covered with bell jars to conserve moisture and kept in a warm greenhouse.

The strawberry plants appeared to be in a sickly condition by the beginning of March with twisting and discolouration of the leaves. One or two of the latter were taken and teased in a little water but no nematodes of any kind were found. There was, however, an abundance of fungal mycelium in the leaf tissues and it seemed clear that the plants were attacked by a parasitic fungus. They gradually became more and more unhealthy and died.

By the time the strawberry runners were first looking unhealthy, the majority of the chrysanthemum cuttings were also showing signs of disease in the leaves which first appeared discoloured and gradually became so badly affected that they dropped off. In all, six cuttings out of the ten originally put in were killed off ; the other four, though showing at first one or two leaves with a little crinkling, have gone ahead and seem to have outgrown the attack.

Examination of the diseased leaves showed them to be very heavily infected with *A. ritzema-bosi* in all stages of growth and there could be no doubt that the latter were the pathogenic agents.

It is interesting to note that although apparently having every opportunity to attack the strawberry plants, the worms did not do so. The results of the experiment may in fact be taken as indicating the physiological specialisation of *A. ritzema-bosi* to the chrysanthemum plant.

Aphelenchus ribes (Taylor, 1917) Goodey, 1923.

Syn. *Tylenchus ribes* Taylor, 1917.

The writer placed this species in the genus Aphelenchus in 1923 on the strength of Taylor's description and figures. In 1926 an opportunity occurred of examining diseased buds of black currant in which numbers of the worm were found and on these a detailed study was carried out. The buds were collected in the vicinity of Cambridge by Mr. E. P. Mumford who kindly sent them to the writer for examination. The worms were examined fresh and after fixation and mounting in weak glycerine. In passing it may be mentioned that a good part of the fresh material was used in an attempt to set an experimental infection of the worm on four young black currant bushes. The teased-up bud material containing active worms was placed in between the scale leaves of dormant buds separated at the time of infection Some of the material was also placed just under the soil close to the stem of each bush. Subsequent examinations during the following season and the year after failed to reveal any sign of disease on any of the bushes nor were any specimens of Aphelenchus ever found in dissected buds taken at various times subsequent to the infection.

The following are the principal measurements :—females, 0·9 to 1·12 mm. long, greatest width, 0·019 to 0·021 mm., anterior end of vulva, 0·66 mm. to 0·74 mm., vulva to anus, 0·28 mm. to 0·31 mm., anterior end to excretory pore, 0·12 mm. to 0·125 mm., stylet, 0·011 to 0·012 mm. ; males, total length 0·9 mm. to 1·1 mm., greatest width, 0·019 mm. to 0·021 mm., spicules, dorsal pieces, 0·022 mm., ventral piece, 0·01 mm., stylet, 0·011 mm. to 0·012 mm. The above measurements show that the worms have practically the same dimensions as *A. ritzema-bosi*. Detailed examination of fresh and fixed specimens shows that they have exactly the same structure as *A. fragariæ* and *A. ritzema-bosi* ; the stylet and its two guides, the œsophagus with the salivary glands, the nerve-ring and the excretory pore are the same in all. The male tail has the three pairs of papillæ and the spicules have the same shape and dimensions as in the two species already described.

Aphelenchus olesistus Ritzema Bos, 1893.

This species is found associated with leaf-blotch in ferns belonging to a number of different genera. It is said also to produce leaf disease in begonias and cypripediums though in view of the fact that larger dimensions are recorded for worms from these hosts (Marcinowski, 1908, p. 408, gives a length up to 1·09 mm. for worms from orchids) it is possible, in the writer's opinion, that she was dealing with *A. ritzema-bosi* or another species.

Ritzema Bos gave the following measurements for worms found in begonia :—females, 0·66 mm. to 0·81 mm., ; males, 0·52 mm. to 0·62 mm. long, and for worms from two species of Asplenium, females, 0·55 mm. to 0·73 mm. long ; males, 0·51 mm. to 0·61 mm. long.

Schwartz (1911) gave the following :—females, 0·497 mm. to 0·644 mm. long ; males, 0·434 mm. to 0·518 mm. long ; figures based on the measurement of worms obtained from ferns belonging to the genus Pteris. These dimensions were quoted in the writer's 1923 paper.

Quite recently the opportunity has arisen for the examination of fresh material of diseased fern fronds of various genera obtained from the Royal Botanic Gardens, Kew, through the kindness of Dr. T. F. Chipp. A fine specimen of leaf-blotched Lygodium sp. was also kindly sent from Edinburgh by Mrs. N. L. Alcock.

Worms were found in *Lygodium* sp.(?), *Lomaria ciliata*, *Blechum brasiliense*, *Blechnum longifolia*, *Nephrodium* sp.(?), *Davallia* sp.(?), *Pteris longifolia* and *Asplenium nidus*. In all cases they were smaller in size than *A. fragariæ*, *A. ritzema-bosi* and *A. ribes* as the following lengths show :—females, 0·57 mm. to 0·76 mm. ; males, 0·47 mm. to 0·56 mm.

As well as being, on the whole, smaller in size than the three species already dealt with, *A. olesistus* presents certain differences in detail from these species. One quite noticeable feature is that the male worms when killed over a flame never die with the caudal region flexed into the marked curve so characteristic of the other species described above. This is shown in fig. 8, where it can be seen that though bent ventrally, the curvature of the tail is only slight compared with that shown by the tails of *A. fragariæ*, *A. ritzema-bosi* and *A. ribes* in figs. 4, 5 and 6.

Another point worthy of note is that the excretory pore is situated in advance of the nerve-ring on a level with or only slightly behind the posterior margin of the muscular bulb of the œsophagus. A word or two on this point is called for in view of a recent opinion expressed by Cobb and Steiner (1927, p. 68). These authorities, in the note before the Helminthological Society of Washington referred to previously, say: "A weak character used to distinguish the various forms is the location of the excretory pore, which obviously often varies in its relative position to the œsophageal bulb because of the latter's mobility."

The writer was at first inclined to agree with this view, but further investigation showed that it is not altogether sound. For example, it has been found that when specimens of *A. olesistus* from ferns are mounted in a drop of water and are carefully killed by heat over a small flame so that they are completely outstretched and have the anterior œsophagus and muscular bulb quite straight, the excretory pore is always found just behind the œsophageal bulb and a little in advance of the nerve ring. On the other hand when examples of *A. fragariæ*, *A. ritzema-bosi* and *A. ribes* are treated in precisely the same way so that the anterior œsophagus is quite straight, the excretory pore is always found some considerable distance posterior to the nerve ring as shown in figs. 1, 2 and 3, which should be compared with fig. 7, of *A. olesistus*. Consequently the writer holds the view that the position of the excretory pore is of some value in attempting to establish specific differences.

Stewart (1921) said that the male of *A. olesistus* had no caudal papillæ but the writer finds that three pairs of papillæ are present in the same positions as in the three species already dealt with.

The spicules have practically the same shape as in those species but are slenderer and smaller. The dorsal limb consists of the usual two pieces forming a hollow tube having a length of 0·014 mm., whilst the single ventral piece has a length of 0·008 mm. On the grounds of smaller size, the position of the excretory pore, and the lesser curvature of the male tail when killed by heat, the writer considers that *A. olesistus* as represented by the forms found associated with leaf-blotch in various kinds of fern, is a species distinct from *A. fragariæ*, *A. ritzema-bosi* and *A. ribes*.

Aphelenchus olesistus var. *longicollis* Schwartz, 1911.

Schwartz found Aphelenchi in some galls on cultivated violets at the bases of the leaf and flower stalks. He was unsuccessful in attempts to set up an infection of healthy violets with the gall material. The following are the principal measurements :—total length—females, 0·483 mm. to 0·728 mm. ; males, 0·525 mm. to 0·637 mm. breadth, 0·014 mm. to 0·015 mm., spicules, dorsal pieces, 0·012 mm. to 0·015 mm., ventral piece, 0·009 mm.

Proportions, $\alpha = 32-35$, $\beta = 7-9$, $\gamma = 13-18$ for females ;
$\alpha = 37-45$, $\beta = 6-8$, $\gamma = 16-19$ for males.

The variety is closely similar to the species but Schwartz considered that it always showed a longer œsophagus, that the excretory pore was always further back from the head end and that the stylet was more heavily built and had more pronounced swellings than in the species.

Aphelenchus cocophilus Cobb, 1919.

Principal measurements :—females, 0·9 mm. to 1·1 mm. long, by 0·013 mm. wide ; males, 0·82 mm. to 1·05 mm. long by 0·009 mm. wide, spicules, dorsal pieces, 0·012 mm., ventral piece, 0·008 mm. Proportions :—females, $\alpha = 83$, $\beta = 20$, $\gamma = 50$; males, $\alpha = 100$, $\beta = 20$, $\gamma = 30$.

As the proportions show the worms are very slender. The female tail tapers gradually to a rather blunt point which is without a terminal process. The male tail, as figured by Cobb, tapers to a fairly sharp point and is markedly coiled towards the ventral surface.

The worms may be said to have the same structural organisation as other species of the genus but it seems a little uncertain whether the stylet possesses basal swellings. There is a well defined muscular œsophageal bulb but the arrangement and posterior extent of the salivary glands are unknown. The female genitalia are similar to those found in other species of the genus, and the vulva is situated at about three quarters of the body-length from the anterior end. The spicules

have the characteristic thorn-shape with the dorsal pieces longer than the ventral one. Cobb says there is a rather obscure accessory piece. With regard to caudal papillæ, Cobb records the presence of one sub-ventral pre-anal and two sub-ventral post-anal in position, the latter being placed fairly close together.

The species is found in the diseased stem and root tissues of coco-nut palms suffering from " red-ring " disease.

Aphelenchus subtenuis Cobb, 1926.

This species has been found in narcissus bulbs in U.S.A., associated with a diseased condition of the leaves and more particularly with the tissues of the upper part of the bulb. In the latter, discoloured areas occur, at first yellow and later turning brown, and in these areas worms of both sexes occur. Usually the number of worms in the diseased tissue is small; mildly diseased material containing two or three nematodes per cubic millimetre, heavily infected tissue, ten or more, and badly infested tissues thousands of worms.

The finding of the worms is the result of extensive field observations on growing plants in the states of Florida, Georgia, Virginia and North and South Carolina. Those plants showing a yellowing or discolouration of the foliage were collected and subsequent examination revealed the presence of the nematode in some of the bulbs. It is suggested that as the worms have been found in bulbs as imported and on plantings of bulbs recently imported, from Europe, the disease is probably of European origin.

Cobb says that experiments have been made which seem to show that the affected bulbs can be satisfactorily treated by the warm-water process as applied to bulbs suffering from attack of *Tylenchus dipsaci*.

Cobb gives the following formulæ for the adult worms:—

Female	1·4	8·6	?	(Med.)	60 70· 15	95·4	0·9 mm.
	1·2	1·8/	?	(2·7)	2·4	1·6	
Male	1·7	8·0	?		80 M	95·7	0.75 mm.
	1·3	2·0/	?		2·4	1·7	

He says that they are closely similar to *A. ritzema-bosi* and suggests that they may even prove to be identical with that species.

The measurements show that they are rather shorter than *A. ritzema-bosi*. The spicules are said to be like those of *A. modestus.*, i.e., *A. parietinus*, and if this is so it is a point of difference from *A. ritzema-bosi*, but possibly Cobb means that in general shape they are like those of the saprophytic species. There are also said to be four (or five) pairs of ventral sub-median caudal papillæ; one pair pre-anal and opposite the proximal end of the spicules; one pair immediately behind the anus; a pair slightly behind the middle of the tail and two closely approximated pairs, often appearing as one, just in front of the terminal caudal process. In possessing a pair of pre-anal papillæ the species differs from *A. ritzema-bosi*, the males of which examined by the writer, had no pre-anal papillæ.

Free-Living Species.

Aphelenchus avenæ Bastian, 1865, type species.

This has already been described in detail in the writer's 1927 paper, but a few remarks are called for on the question of its identity with *A. agricola* de Man, 1884. On p. 210 *A. agricola* is given as a synonym of *A. avenæ*, a view also adopted by Micoletzky (1921). After reading this paper, Dr. N. A. Cobb was good enough to send the writer some notes on this matter. He would apparently keep the species separate on the following grounds:—1, Bastian says nothing in his original description of *avenæ*, about the presence of longitudinal striæ on the lateral fields, whereas de Man figures them in *agricola*. 2, Difference in habitat; *avenæ* was found by Bastian between the leaf-sheaths of oats and the forms which Cobb has found and identified as *avenæ* have also come from inside plants and have been without longitudinal striæ on the lateral fields. *A. agricola*, on the other hand, was found by de Man in the vicinity of roots of plants growing on sand dunes in Holland and Cobb says he has numerous records of this species taken from about the roots of plants.

The specimens examined by the writer, as stated in the previous paper, were from decaying plant tissues and one would assume, according to the habitat criterion, that they should be *avenæ*. A careful examination was therefore made of the mounted worms to determine whether or no there were longitudinal striæ on the lateral fields with the result

that in specimens obtained from a diseased potato tuber, fine longitudinal striæ were found on the lateral fields quite distinct in appearance from the coarse longitudinal striations seen underlying the cuticle in other parts of the body. This goes to show that although not found in the region of roots the worms agree with *A. agricola* in a detail of appearance and is additional evidence in support of the view that the two species should be considered as identical.

The *A. agricola* described by Maupas (1900), p. 571, is also, in the writer's opinion, the same as *A. avenæ*. Micoletzky (1921), p. 603, gave it a new specific name, *A. Maupasi*, and placed it in his sub-genus *Paraphelenchus* mainly because of the presence of a somewhat Tylenchus-like post-bulbar portion of the œsophagus. This was scarcely necessary as the worms agree with *A. avenæ* in this feature of their anatomy, and also in the shape of the head and tail, in the stylet and in having fine longitudinal striations on the lateral fields.

Aphelenchus parietinus Bastian, 1865.

Syn. *A. coffeæ* Zimmerman, 1898; *A. erraticus* Linstow, 1876; *A. littoralis* Hofmänner, 1915; *A. microlaimis* Cobb, 1893; *A. minor* Cobb, 1893; *A. modestus* de Man, 1876; *A. ormerodis* Ritzema Bos, 1891; *A. penardi* Steiner, 1914; *A. pyri* Bastian, 1865; *A. richtersi* Steiner, 1914; *A. rivalis* Bütschli, 1873; *A. striatus* Steiner, 1914; *A. villosus* Bastian, 1865.

Micoletzky (1921), p. 590, gives the following measurements:— female, length, 0·62 mm. (0·35 – 1·05), $\alpha = 31·6$ (23 – 43), $\beta = 10$ (7 – 15), $\gamma = 15$ (11 – 21), vulva 70 per cent. (66 – 78) of body length from anterior end; male, length, 0·605 mm. (0·35 – 0·9), $\alpha = 34·3$ (25 – 47), $\beta = 10$ (8 – 16), $\gamma = 15·5$ (10 – 23). Specimens examined by the writer have given the following:—
females, length, 0·65 mm. – 0·8 mm., $\alpha = 28 – 38$, $\beta = 9 – 11$, $\gamma = 14 – 15$;
males, length, 0·57 mm. – 0·75 mm., $\alpha = 25 – 30$, $\beta = 9 – 11$, $\gamma = 14 – 15$.

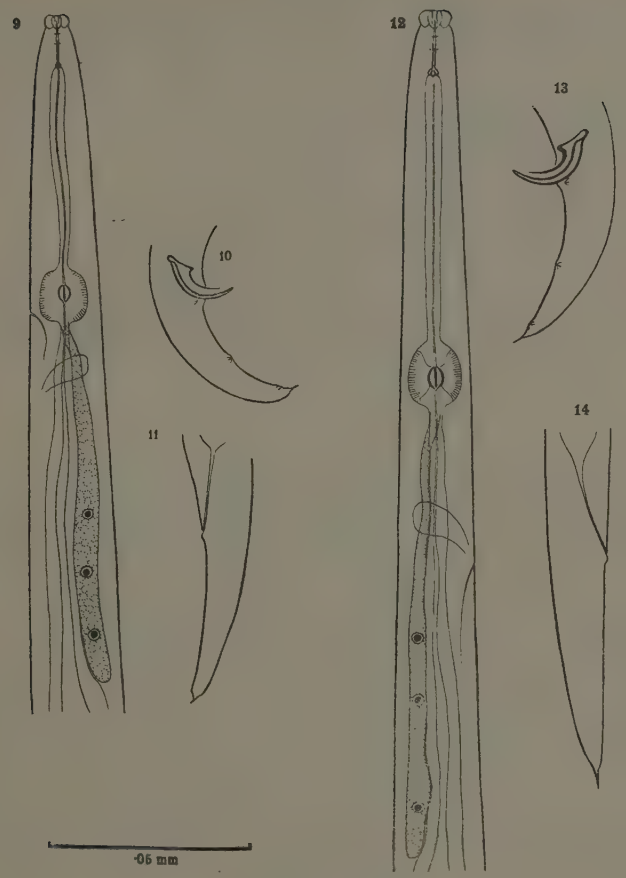
This species is probably one of the commonest and most widely distributed of free-living saprophytic nematodes as the above list of synonyms alone would suggest. The writer has found it in washings from pasture grass along with *A. helophilus* and in decaying vegetable matter; potato tuber, freesia bulb, mint rhizome and pea roots in association with other saprophytic nematodes.

Cobb (1927), p. 71, in a recent note is apparently inclined to the view that it is parasitic for he speaks of it as "a rather common parasite of narcissus bulbs in the extreme north-western part of United States." Experimental investigations on this point would be valuable especially in view of the widespread occurrence of the species.

The head is, as a rule, distinctly knobbed with convex sides as in *A. fragariæ* and the outlines of the six lips fused together can be made out by focusing on the surface. The swellings at the base of the stylet are not prominent and do not extend across the lumen, appearing rather as outer lateral thickenings of the wall. The œsophagus is of the usual type and calls for no description. In specimens in which the first part of the œsophagus and the bulb are straight, the excretory pore is in advance of the nerve-ring and just behind the posterior rim of the muscular bulb. The salivary glands have the same arrangement as in *avenæ* and *fragariæ*. The female genitalia are of the same type as in *avenæ*. The vulva is situated at approximately three-quarters of the body length form the anterior end. The ovary extends almost as far as the muscular bulb and is occasionally reflexed. There is a post-vulvar uterine sac. The male gonad is single and is also occasionally reflexed anteriorly.

The male tail has been described in the preceding paper, pp. 214 and 215. It is there shown that there are three pairs of caudal papillæ; one pair ad-anal and two pairs post-anal in position of which one pair is almost terminal and the other pair about half way between the anus and the tip of the tail. The spicules have been dealt with in the earlier paper (1927) and are very similar to those in *fragariæ*. The drawing of them, fig. 9 of that paper, is not strictly correct in that it does not show the inner portion of the dorsal pieces connected with the outer piece; it is more accurately figured in fig. 10.

The tip of the tail in both sexes is usually furnished with small irregularities and a pointed terminal process of variable size. On account of the variability of the tail, Micoletzky (1921), proposes a classification of the species. Thus, forms with a well-defined, long terminal process come into variety *tubifer* whilst those with a very small process come into *microtubifer*. Each variety again is further classified according to the size of the body; those greater than 0·7 mm., females and 0·6 mm., males are form *magnus* and those shorter than this are *parvus*. Each



Aphelenchus parietinus.

Figs. 9, 10 and 11.—Anterior end, male tail and female tail respectively. Note the excretory pore in advance of the nerve ring and the small swellings at the base of the stylet.

Aphelenchus helophilus.

Figs. 12, 13 and 14.—Anterior end, male tail and female tail respectively. Note excretory pore well behind the nerve ring and the well-marked basal swellings of the stylet.

of these again is further defined according to the degree of body slenderness, into sub-form *gracilis* or *informis*. This gives a scheme carried to the fourth degree, species, variety, form and sub-form. It is doubtful, in the writer's opinion, how far such a refinement of classification can be considered of scientific value, for we do not know to what extent these small differences are constant for the species.

Aphelenchus helophilus de Man, 1880.

Micoletzky (1921) gives the following as synonyms :—

A. sp. Ditlevsen, 1911. *A. elegans*, Micoletzky, 1914.

The species is chiefly distinguished from *parietinus* by being longer and slenderer and by the tail tapering to a fine single process, not plump and rounded. The stylet is also said to be rather heavy in structure with well marked basal swellings. de Man found the worm twice in damp earth in the region of grass roots ; the male in May and the female in February.

The writer has found numerous examples of what he takes to be *A. helophilus* on several occasions in water extractions made by the Baermann method, from grass blades taken from a pasture at Winches Farm.

The following are measurements given by de Man (1884), p. 140 :— Length, female, 1 mm., male 0·8 mm., $\alpha = 55 - 65$, $\beta = 10 - 12$, $\gamma = 14$ female, 20 male. Micoletzky (1921) gives length, female 0·87 mm. to 1·4 mm., $\alpha = 46 - 78$, $\beta = 10 - 17$ $\gamma = 14 - 17{\cdot}8$, vulva 65 per cent. to 66 per cent. of body length from anterior end. The worms examined by the writer have given the following measurements :—Females 0·85 mm. to 1·3 mm., males 0·8 mm. to 1·1 mm. ; proportions, females, $\alpha = 45 - 52$, $\beta = 9 - 10$, $\gamma = 16 - 20$; males, $\alpha = 43 - 55$, $\beta = 9 - 10$, $\gamma = 20$. These agree fairly well with those of de Man and Micoletzky. The worms are very similar to *A. parietinus* in appearance and structure. The principal differences noticed between them, in addition to the greater length and slenderer character of *A. helophilus*, are as follows :—

1. In *A. helophilus* the stylet has more pronounced basal swellings than in *A. parietinus*.

2. The excretory pore is situated some considerable distance posterior to the muscular œsophageal bulb and behind the level of the nerve-ring, whereas in *A. parietinus* it is in advance of the latter.

3. The tip of the female tail, though very similar to that of *A. parietinus*, has the terminal process more gradually produced to a fine tapering process.

4. The spicules, though of the same shape as those of *A. parietinus*, are rather stouter in appearance and the dorsal pieces have a greater length, being 0·026 mm., as compared with 0·022 mm., in *A. parietinus*.

All the above differences are admittedly small and might be considered by some as scarcely warranting the separation of the two species, but when once worms are seen side by side the greater length and slenderer character of *A. helophilus* are apparent and these features, coupled with the larger basal swellings of the stylet and the posterior position of the excretory pore, justify one in keeping the species distinct.

Aphelenchus pseudolesistus n. sp.

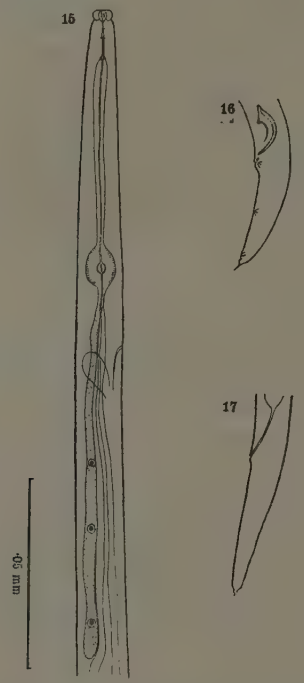
The writer has obtained representatives of this new species on two occasions, first from washing of decaying oak leaves taken from a ditch at Winches Farm in February, 1928, and secondly from teasings and washings of a gall-formation on the root-stock of *Chrysanthemum maximum* sent to this Institute for examination in May, 1928.

Principal measurements:—females, length, 0·72 mm. to 0·87 mm., $\alpha = 58-62$, $\beta = 9-10$, $\gamma = 15-16$; males, length, 0·52 mm. to 0·66 mm., $\alpha = 53-55$, $\beta = 9-10$, $\gamma = 20$. The worms are very slender and delicate in appearance and have a very small œsophageal bulb. They very closely resemble *A. olesistus* in all features, except that they are longer, and for this reason the specific name *psuedolesistus* has been chosen for them.

The excretory pore is situated only a short distance behind the œsophageal bulb and in advance of the nerve-ring as in *olesistus*. The vulva is found at about two-thirds of the body length from the anterior end and the vagina leads obliquely forwards. The gonad is of the usual type and there is a post-vulvar uterine sac extending to a little more than half the distance between the vulva and the anus.

The fact that Schwartz described his *A. olesistus* var *longicollis* from galls on a root-stock of violets has not escaped the writer's attention and the present species would possibly have been referred to that

variety but for the fact that it was obtained from decaying oak leaves in an open ditch as well as from the galls on *Chrysanthemum maximum* which suggests that it is probably a saprophytic species only.



Aphelenchus pseudolesistus n. sp.

Figs. 15, 16 and 17.—Anterior end, male tail and female tail respectively. Magnification same as for figs. 9 to 14.

The male tail is also very similar to that of *olesistus* in that it is not markedly curved ventrally after fixation by heat. The spicules and arrangement of the caudal papillæ are exactly as in *olesistus*.

Aphelenchus tenuicaudatus de Man, 1895.

This species was obtained by de Man from rotting pseudo-bulbs of orchids, hybrid Calanthes, sent from the greenhouses of the Duke of Westminster at Chester; the original plants from which they were propagated having come from Veitch's of Chelsea. The original description was based on the examination of numerous specimens of adults of both sexes.

In February, 1928, the writer found three examples of the species, two males and one female, along with other nematodes in rotting banana rhizome which had been growing in a hot-house at the Royal Botanic Gardens, Kew, and was sent, preserved in alcohol, by Dr. E. J. Butler, of the Imperial Bureau of Mycology. The worms were in a good state of preservation, so that after transference to glycerine alcohol and subsequent mounting in weak glycerine a good deal of their structure could be made out.

Since de Man's original description, the species had not been recorded again until quite recently when Steiner (1927*a*) found it in decaying orchid material, *Cattleya* species, sent from New York. He only obtained female examples, and concludes that the male is probably scarcer than the females. He also suggests that the species is probably peculiar to orchids since his and the original findings were from orchids. The fact that the writer has obtained the species from banana tissues shows that it is not peculiar to orchids. Both orchid and banana plant from which the worms have, so far, been obtained have been grown in greenhouses, and the writer would suggest that rather than being associated with a particular host plant the worms are more probably connected, in some way, with the special soils or composts used in the cultivation of plants under warm greenhouse conditions.

Principal measurements :—de Man gives the following, length, female, 0·95 mm., male 0·8 mm., proportions, female $\alpha = 35 - 36$, $\beta = 9 - 9\frac{1}{2}$, $\gamma = 7\frac{3}{4} - 8\frac{3}{4}$, male $\alpha = 35 - 36$, $\beta = 8\frac{1}{2} - 9$, $\gamma = 11 - 15$.

The writer obtained the following measurements and proportions :— Female, length, 0·67 mm., $\alpha = 32$, $\beta = 7 \cdot 4$, $\gamma = 8$; male, length, 0·6 mm., $\alpha = 34$, $\beta = 7 \cdot 5$, $\gamma = 11$. Steiner gives the length of two females as 0·58 mm. and 0·62 mm. with other proportions in Cobb's formula which agree well with the dimensions indicated above.

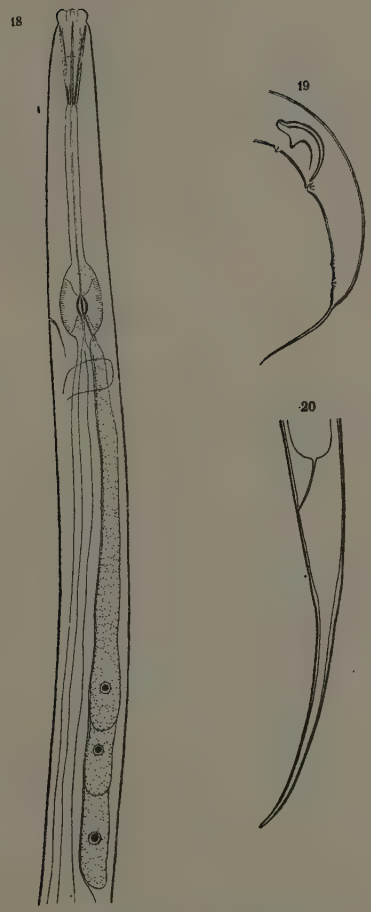
The head is furnished with six distinct rounded lips which are without papillæ. In the writer's examples each lateral lip seemed to possess a small depression which is considered to be the aperture of the amphid, as from it could be seen two lines passing backwards which would correspond in position to the duct of the amphid. The stylet is long, sharply pointed and without basal swellings. de Man gives the length as 0·024 mm. to 0·027 mm., Steiner as 0·022 mm. to 0·026 mm., the writer's measurements give 0·022 mm. There appears to be a spear collar or guide about halfway down the sylet in the writer's specimens. A conspicuous feature at the anterior end of the worm is the muscles attached to the base of the stylet. The narrow first part of the œsophagus leads to the muscular bulb which is elliptical in outline and from one and a half to twice as long as broad. In the writer's specimens the bulb showed a broad wedge of granular tissue anteriorly and posteriorly, very different in appearance from the muscular material of the central region. Steiner mentions the same feature in the worms studied by him.

The excretory pore is rather variable in position; de Man figures it behind the œsophageal bulb, Steiner level with the anterior margin of the latter, and the writer has found it about midway of the length of the bulb. It is, of course, in advance of the nerve-ring.

The salivary glands are very large and extend for a considerable distance posterior to the œsophageal bulb. de Man figured them, but did not show their connection with the alimentary tract. Steiner speaks of a single long salivary gland, but the writer finds distinct indications of three glands in the large mass of tissue lying dorso-laterally to the first part of the intestine. A duct or ducts seem to lead from the anterior end of the glands through the posterior granular wedge of material of the œsophageal bulb to the lumen of the œsophagus. Steiner mentions the same point also.

The vulva is situated between one quarter to one-third of the body length from the tip of the tail; the gonad is single and extends anteriorly almost as far as the posterior end of the salivary glands. There is a long and clearly defined post-vulvar uterine sac figured by de Man and also easily seen in the writer's specimen.

The male gonad is single and in the writer's specimens the anterior end is not reflexed; de Man says that in some of his it seemed to be



Aphelenchus tenuicaudatus.

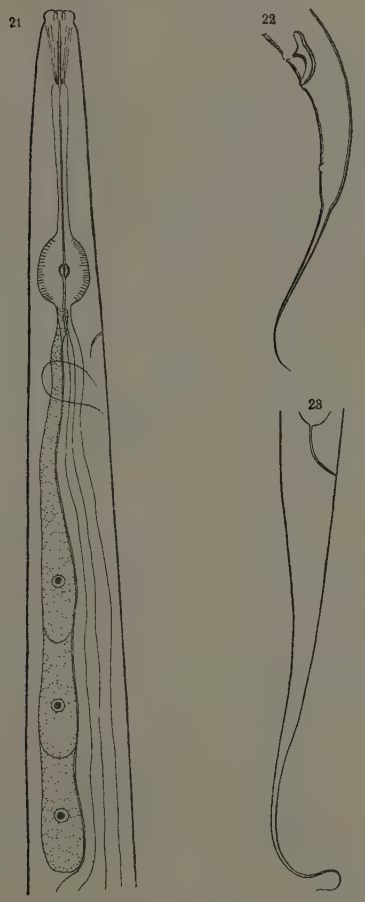
Figs. 18, 19 and 20.—Anterior end, male tail and female tail respectively.

reflexed for a short distance. The spicules have the characteristic thorn-shape with the anterior ends rather broad, as figured by de Man. After careful examination under oil-immersion it seems that the dorsal limb of each is made up of two pieces as in most of the other species of the genus examined by the writer. The arrangement of the male caudal papillæ as described and figured by de Man has been found by the writer also. There is a single pair of pre-anal papaillæ and two pairs of post-anal anal papillæ some distance posterior to the anal opening ; all are ventro-lateral in position. In one of the specimens examined by the writer there also appeared to be a rather obscure ad-anal papillæ, such as is found in *A. parietinus* and the plant-parasitic species already dealt with.

Aphelenchus demani n. sp.

This species, in possessing a long, finely pointed tail, is closely similar to *A. tenuicaudatus*, *A. longicaudatus* and *A. winchesi* from each of which it can be separated on certain details of structure. The writer has named it in honour of Dr. J. G. de Man, as he was undoubtedly the first to make observations on it. The writer sent word to Dr. de Man of finding *A. tenuicaudatus* in banana material, and in his reply the Dutch worker very kindly sent notes and tracings of drawings of female worms which he had found in diseased hop-roots sent to him by Ritzema Bos who had received them from Mr. Duffield of the South Eastern Agricultural College, Wye, in 1915. Early in May, 1928, the writer found seven or eight females and one male of the same species amongst other nematodes which had climbed up some grass blades from a turf of grass which had been cut from a pasture and placed in a glass cylinder covered with a plate of glass. The tips of several blades of grass had come into contact with the drops of water condensed on the under side of the lid and the worms were found in these drops. Drawings and measurements of the worms were made after carefully killing over a small flame and when these were compared with the tracings and measurements sent by Dr. de Man it was evident that the same species had been found.

The writer, thereupon, wrote to inform him of the discovery of the worms, at the same time suggesting the name *demani* for the species if Dr. de Man would agree. The latter replied expressing agreement to the name, and at the same time enclosing further particulars on



Aphelenchus demani n. sp.

Figs. 21, 22 and 23.—Anterior end, male tail and female tail respectively. Same magnification as for figs. 9 to 14.

two females and one male of the same species which he had found in 1916 in a diseased Narcissus bulb which had been sent to him by Ritzema Bos from a district south of Haarlem. Drawings of the male were not made, but only measurements taken.

A word or two more on the occurrence of the worm may be given here. Amongst specimens of *A. parietinus* now mounted in weak glycerine taken from a diseased potato tuber in 1926, the writer noted three or four female worms with long tapering tails which at the time were considered as probably belonging to a new species. These have now been examined again and prove to be members of the same species as shown by their size, the shape of the tail and the length and characters of the stylet. The full list of the occurrence of the species up to date is therefore as follows :—Hop-roots, England, de Man, 1915 ; narcissus bulb, Holland, de Man, 1916 ; potato tuber, England, writer, 1926 ; grass blades, England, writer, 1928. The writer is greatly indebted to Dr. de Man for his kindness in sending his notes and measurements, which go to make our knowledge of the worms more complete.

Principal measurements :—Female, length, 0·64 mm. to 0·76 mm., breadth, 0·022 mm. to 0·025 mm., anterior end to end of œsophageal bulb, 0·065 mm. to 0·07 mm., anus to tip of tail, 0·12 mm. to 0·14 mm., stylet, 0·0166 mm., proportions, $\alpha = 32-34$, $\beta = 9-11$, $\gamma = 5{\cdot}3-5{\cdot}8$; male, length, 0·53 mm., breadth, 0·016 mm., anterior end to end of œsophageal bulb, 0·064 mm., anus to tip of tail, 0·08 mm., stylet, 0·0162 mm., spicule, dorsal pieces, 0·016 mm., ventral piece, 0·009 mm., proportions, $\alpha = 33$, $\beta = 8{\cdot}3$, $\gamma = 6{\cdot}6$.

The following are measurements supplied by Dr. de Man of worms examined by him in 1915 :—female, 0·6 mm. long, tail 0·06 mm. long ; $\alpha = 33$, $\beta = 9$, $\gamma = 10$; female, 0·7 mm. long, tail 0·127 mm. long, $\alpha = 35$, $\beta = 9{\cdot}4$, $\gamma = 6{\cdot}5$, stylet 0·0186 mm. long. In 1916 female, 0·726 mm. long, $\alpha = 35$, $\beta = 9{\cdot}4$, $\gamma = 5{\cdot}7$, stylet 0·0192 mm. ; male 0·545 mm. long, $\alpha = 40$, $\beta = 8$, $\gamma = 7$, stylet 0·0162 mm. It will be noticed that the length of the stylet as given by de Man is longer, except in the case of the male, than given by the writer. This is probably to be accounted for by assuming that the higher numbers represent the distance from the anterior end of the worm to the base of the stylet,

whereas the writer's measurements are taken from the tip of the stylet to its base on high-power drawings.

The head end is set off from the body and has six rounded lips which are not so clearly defined, however, as are those of *A. tenuicaudatus*. The stylet is comparatively long but not so long as that of *A. tenuicaudatus* and *A. winchesi*. It is made up of an anterior conical part which is a little more than half the length of the succeeding cylindrical part. Its walls are rather thin and the basal swellings only appear as small thickenings on the outside of the tube; they do not extend across its lumen. The anterior part of the œsophagus and the muscular bulb are of the usual type. The bulb is a little longer than broad and is narrower anterior to the central valve than posterior to it.

The three salivary glands are large and prominent as in *A. tenuicaudatus* and *A. winchesi* and occupy the same relative position in the body. The excretory pore is found a short distance behind the bulb and in advance of the nerve-ring. The vulva is situated about two-thirds of the body length from the anterior end. The vagina has stout walls and the gonad is single and directed anteriorly. There does not appear to be a post-vulvar uterine sac and in this respect it resembles *A. winchesi* and differs from *A. tenuicaudatus*.

The tail is long and tapers to a fine thread-like point which appears to be easily broken off; a fact which would account for the variable lengths given above.

The male tail is very similar in appearance to that of both *A. tenuicaudatus* and *A. winchesi*. The long finely tapering termination is longer than that of *A. tenuicaudatus* and slenderer that the corresponding part in *A. winchesi*. The spicules are practically identical in shape with those of *A. winchesi* and are smaller than those of *A. tenuicaudatus*. In possessing one pair of pre-anal papillæ it resembles *A. tenuicaudatus* and dfffers from *A. winchesi* whilst in having only one pair of post-anal papillæ it resembles *A. winchesi* and differs from *A. tenuicaudatus* which has two pairs. The writer could not determine whether a pair of ad-anal papillæ was also present.

It is probable that the single female worm referred by Micoletzky (1921), p. 601, to *A. tenuicaudatus* should be transferred to the species under description for it is figured with a long stylet having small basal swellings. The head, however, appears to be without lips.

Aphelenchus longicaudatus Cobb, 1893.

This species, which also has a long tapering tail, was found by Cobb in small numbers about the roots of banana plants in Fiji. He gives the following formulæ :—

Female	2·6	11·0	10·0—55·0 [40]		70·0	0·8 mm.
	1·6	1·9	1·8	2·2	1·1	
Male	3·0	14·0	13·0 — M		72·0	0·57 mm.
	1·4	2.0	2·0	2·6	1·0	

The following is taken from the original brief description. " Lips obscure, probably six ; spear acute, slender, with inconspicuous posterior ending ; tail, conical, in its posterior half setaceous ; vulva inconspicuous ; uterus with possibly a rudimentary posterior branch ; the male tail is like that of the female in form. There is no bursa or papilla or supplementary organ of any kind. The arcuate cuneiform spicula are a little longer than the anal body-diameter ; their proximal ends not being cephaloid. There is a chitinous structure in front of the spicula, possibly the chitinous terminal portion of the combined rectum and sexual opening, which is half as long as the spicula and expanded proximally much as in *A. microlaimus*." The last-mentioned chitinous structure is, in the writer's opinion, undoubtedly the ventral piece of the spicules, as is all the more evident when the drawing is compared with that of *A. microlaimus*, where a similar structure is said to occur. Apparently in neither species was the bridging portion between dorsal and ventral pieces made out.

With regard to the status of this species it is obviously closely related to *A. demani*, *A. tenuicaudatus* and *A. winchesi*, particularly in the shape of the tail, but in the absence of any exact information as to whether the stylet has basal swellings and because the male tail is said to lack papillæ it seems advisable to retain *A. longicaudatus* as a distinct species.

Aphelenchus chamelocephalus Steiner, 1926.

This species, described from female specimens only, obtained from diseased South African peanut plants, is closely similar to *A. parietinus*.

The following formulæ are given for two females :—

$$\frac{2\cdot1 \quad ? \quad ?}{2\cdot4 \quad ? \quad ?} \quad {}^{35}\frac{68\cdot0 \quad 93\cdot0}{3\cdot8 \quad 2\cdot1} \quad 0\cdot507 \text{ mm.}$$

$$\frac{1\cdot5 \quad 12\cdot0 \quad ?}{1\cdot5 \quad 3\cdot1 \quad ?} \quad {}^{51}\frac{71\cdot0 \quad 94\cdot5}{3\cdot1 \quad 1\cdot9} \quad 0\cdot546 \text{ mm.}$$

The cuticle has fine transverse striations which do not cross the lateral fields. Steiner separates the species from *parietinus* on the grounds that the head is not set off from the body like a button but is bluntly conical in shape and because the stylet is obsolescent, being only just discernible under very high magnification and apparently is without basal swellings.

The figures show the nerve-ring just posterior to the œsophageal bulb and the excretory pore behind the nerve-ring. There are three salivary glands in the usual situation. The gonad is single and outstretched anteriorly and there is no post-vulvar uterine sac.

Aphelenchus retusus Cobb, 1927.

Formula :— $\frac{2\cdot2 \quad 13\cdot0 - ?}{1\cdot8 \quad 3\cdot4_{j} - ?} \quad {}^{50}\frac{77\cdot0 \quad 96\cdot1}{3\cdot5 \quad 2\cdot5} \quad 0\cdot72 \text{ mm.}$

Cobb says that in form the species is most like *A. neglectus* Rensch, and *A. dubius* Steiner, and calls attention to three points in which it shows differences from *A. dubius* var *peruviensis* Steiner :—1, the striæ are finer ; 2, the spear is non-bulbous ; 3, the tail is sub-cylindroid, its terminus sub-hemispherical with a nearly central dimple. One egg in the uterus at a time apparently laid before segmentation, body a little narrower immediately behind vulva. Male unknown but syngonic sperms present in the uterus just in front of the egg.

Habitat : Present in a dead pupa of a fly *Chætopsis ænea*, from Milford, Iowa, U.S.A.

In view of the fact that Cobb calls attention to the likeness of the worms to *A. dubius* and *A. neglectus* neither of which is a valid species of Aphelenchus it seems a little doubtful to the writer whether the present species should be considered as belonging to Aphelenchus also. No drawings are available from which to form an opinion.

Aphelenchus gœldii Steiner, 1914.

The species occurred in damp humus. The dimensions given are as follows:—length 0·331 mm., breadth 0·014 mm., œsophagus 0·054 mm., tail 0·025 mm., proportions $\alpha = 23·6$, $\beta = 6·1$, $\gamma = 13·2$.

The body is very narrow, tapering a little anteriorly but more posteriorly. Cuticle finely striated; head end not set off from the body, without bristles and lips but with six papillæ. Stylet very delicate, weakly knobbed posteriorly appearing to be of the Tylencholaimus type, *i.e.*, the posterior part is not fused into a single tube. Œsophagus narrow, bulb oval with three chitinous thickenings. Location of nerve ring and excretory pore uncertain. Vulva two-thirds of body length from anterior end, gonad single, anterior, no post-vulvar sac. Tail short, gradually narrowing and ending in a fine gland outlet tube. Male unknown.

Micoletzky (1921), p. 590 includes this species, as a synonym of *A. parietinus*, but whether he is justified in so doing seems a little doubtful to the writer in view of its rather peculiar head characters and the fact that Steiner says definitely that there is no post-vulvar sac.

Aphelenchus naticochensis Steiner, 1920.

This species is based on two female worms collected from Lake Naticocha, Peru. The following dimensions are given:—0·774 mm. long by 0·012 mm. wide and 0·911 mm. long by 0·014 mm. wide; proportions $\alpha = 63·2$, $\beta = 11·9$ and 13·3, $\gamma = 23·9$ and 31·6.

Body very slender and slightly narrower at anterior and posterior ends. Cuticle very finely striated; head end not set off and anteriorly rounded, papillæ absent and distinct lips not visible. Tail short, with small terminal process; vulva at about three-quarters of body length from anterior end. Stylet very delicate and scarcely visible, apparently unknobbed. Œsophageal bulb pear-shaped, excretory pore posterior to nerve-ring, gonad single and anterior. The species is very similar to *A. parietinus* but differs in the character of the head, the stylet, the shape of the œsophageal bulb and in having the excretory pore behind the nerve-ring.

Aphelenchus caprifici (Gasparrini, 1871) Cobb, 1927.

This species is very briefly referred to by Cobb (1927), p. 57, as having the onchium, *i.e.*, the stylet, distinctly cleft at the base. He proposes it as the type of a new sub-genus *Schistonchus*.

As this was the only mention of the species know to the writer he wrote to Dr. Cobb who very kindly sent the reference to the work of Gasparrini and also copies of his own unpublished notes and photographs of his original drawings of the worms. From a study of these it is evident that the worms are true species of the genus Aphelenchus as revealed by the character of the first part of the œsophagus, the muscular œsophageal bulb, the arrangement of the male caudal papillæ and the shape of the spicules.

The most noticeable feature of the anatomy is the stylet which is bifurcated for about the last third of its length so that it has somewhat the appearance of an inverted letter Y with a slight swelling at the base of each limb.

The dimensions given in Dr. Cobb's notes show that the worms are quite small; the length of female and male being given as 0·5 mm. and 0·4 mm. respectively.

Habitat: Living in between the overlapping segments of the abdomen of the fig-wasp *Blastoghaga* within fig fruits and apparently utilising the wasp as a means of transport. The specimens examined by Dr. Cobb came from Algiers.

Species Inquirendæ.

Aphelenchus aderholdi Schwartz, 1912.

Worms ascribed to this species were found in diseased Lily-of-the-Valley roots sent from Ervitz, Mecklenburg-Schwerin. The roots showed copper coloured and carmine spots as previously observed by Aderhold in 1900 on diseased roots of the same kind of plant. It was not possible to identify the worms with any previously described species of Aphelenchus nor from the material at hand was it possible to determine whether the worms were responsible for the disease. A brief diagnosis

of the species is given with the following dimensions and proportions: —female 0·728 mm. to 1·216 mm. long, $a=32-58$, $\beta=11-15$, $\gamma=13-19$, body length/distance of vulva from anterior end = 2·8 – 3·2, œsophagus length/stylet length = 7 – 11. Tail conical to a point without papillæ, with a terminal process, cuticle smooth, head without papillæ, stylet knobbed.

Male 0·88 mm. long, $a=42$, $\beta=13$, $\gamma=24$, body length/distance of excretory pore from anterior end = 8, œsophagus length/stylet length = 8. Tail conical to a point with a terminal process with a lateral papilla about midway of the tail, a papilla between the first papilla and the end of the tail, and a median papilla near the end of the tail. Cuticle smooth, head without papillæ, stylet knobbed.

In the absence of drawings of the worms and in view of the almost entirely negative characters given in the description it is impossible to form an opinion as to the relationships of this species. The lengths given are rather long for *A. parietinus* and are nearer to those of *A. helophilus*, whilst the papillæ of the male tail may be considered as having the same arrangement as those in *A. parietinus*.

Aphelenchus mycogenes Schwartz, 1912.

This species was found in a culture of a fungus *Cryptosporium nesii* five and a half months after it had been set up, possibly through the use of some unsterilised tap water. Schwartz says he could not identify it with any previously described form and therefore erects a new species for its reception. Tail conical to a point, without papillæ with terminal process, cuticle smooth, head without papillæ and stylet knobbed. 0·4 mm. to 0·683 mm. long, $a=22-32$, $\beta=7-11$, $\gamma=12-19$, body length/distance of vulva from anterior end = 2·9 – 4·2, œsophagus length/stylet length = 7 – 11. Male unknown.

Possibly the worms should be transferred to *A. parietinus*.

Aphelenchus coffeæ Noack, 1898.

Particulars concerning this are so scanty that it is impossible to say whether it belongs to the genus or not.

Species wrongly attributed to Aphelenchus.

Aphelenchus neglectus Rensch, 1924.

Rensch described worms obtained from the cortex of the roots of barley, wheat, and certain grasses as *A. neglectus*; claiming that it should be classed in this genus because there was no second part to the œsophagus such as occurs in members of the genus Tylenchus. He found only females and larval forms.

Goffart (1927) has produced a short paper on the species and deals mainly with the question of its parasitism on a wide range of host plants of economic importance including barley, oats, wheat, rye, sugar-beet, rape, various varieties of cabbage, mustard and peas.

The systematic position of the species was doubtful because neither Rensch's nor Goffart's descriptions and figures clearly revealed the structure and organisation of the post-bulbar region of the alimentary canal. Steiner (1927) in a note discussing injury to plants due to *Tylenchus pratensis* de Man, gives *A. neglectus* as a synonym of that species. The note has been followed by a paper (Steiner, 1927*a*) in which he shows conclusively that *A. neglectus* should be considered as a synonym of *Tylenchus pratensis* de Man, 1884, with which also *Tylenchus penetrans* Cobb, 1917, is said to be synonymous. The species possesses a post-bulbar glandular œsophagus which is rather difficult to define. Steiner also confirms the fact that it can parasitise the roots or a number of cultivated plants and is consequently of considerable economic importance.

Aphelenchus fœtidus Bütschli, 1874.

This species was originally found in cow-dung. In listing the various species of Aphelenchus described up to date, Cobb (1891) gave it as his opinion that the species does not belong to the genus. He evidently was in doubt about the structure of the stylet and concludes as follows, "If there is no doubt about the spear, a new genus will probably have to be created for it."

In the writer's opinion also the worms do not belong to the genus Aphelenchus at all. Bütschli himself recognised that they presented certain characters which distinguished them from both Tylenchus and

Aphelenchus ; from the former chiefly in the absence of a male bursa and from the latter in possessing a posterior œsophageal bulb. He also pointed out that the male, in possessing a number of caudal papillæ, differed from both genera. Micoletzky (1921) placed the species in his sub-genus Paraphelenchus, but there is no justification for such a procedure.

The species should be placed in the genus *Tylopharynx* de Man. This conclusion is based on a comparison of Bütschli's original drawings with those of *Tylopharynx striata* given by de Man (1876 and 1884) and with drawings made by the writer of this interesting species which was found in soil from a chicken-run at Winches Farm. The points of resemblance are as follows :—

1. *Shape of the Anterior End.* Bütschli's fig. 5*a* gives a lateral view of the fore part of a worm and shows two distinct angular prominences one on either side of the extreme anterior end. These two points are very characteristic of *Tylopharynx striata* as the writer's observations have proved to him. They are only seen when the worm is viewed in lateral aspect.

2. *Head Papillæ.* Bütschli considered that there were six anterior papillæ on the head ; a feature in which his worms differed from both Tylenchus and Aphelenchus. The writer finds that the examples of Tylopharynx studied by him possess six small head papillæ.

3. *Stylet.* The buccal spear figured by Bütschli has basal swellings, but the description of the worm contains no detailed account of this structure, and it is impossible to determine whether it is like that frequently found in Tylenchus and Aphelenchus or like that of Tylopharynx, which has a very different and complex make-up but has the *appearance* of a typical hollow stylet with basal swellings even under moderately high magnification.

4. *Œsophagus.* The œsophagus figured by Bütschli is similar in appearance to that of Tylopharynx with a prominent muscular bulb. He also shows a second glandular portion of the œsophagus such as is found in Tylopharynx.

5. *Female Gonad.* This, according to Bütschli is paired, opposed and reflexed in *A. fœtidus.* In this it differs from most Aphelenchi but resembles Tylopharynx in which also the vulva is situated about midway of the body.

6. *Spicules and Gubernaculum.* It is in respect to these characters and the arrangement of the male caudal papillæ that the strongest resemblance is shown to Tylopharynx. The spicule figured by Bütschli is quite unlike that found in Aphelenchi but closely similar to that of Tylopharynx as found by the writer. It is long, tapers to a fine point and has the anterior end knobbed. There is also a well developed gubernaculum which is not found in Aphelenchi but is a prominent feature in Tylopharynx.

7. *Male Caudal Papillæ.* These are numerous and have a different distribution from those found in Aphelenchus. The arrangement figured by Bütschli is closely similar to that found in Tylopharynx studied by the writer which is on exactly the same plan as that found in males of Diplogaster, Odontopharynx and Cylindrogaster. In the lateral series the papillæ have the same disposition in *A. fœtidus* as in Tylopharynx; one pair pre-anal, one pair about midway between the level of the anus and the beginning of the tail, and one pair rather dorsally situated just at the base of the tail. Bütschli shows four small papillæ close together, posteriorly placed, very close to the mid-ventral line. There are really only three papillæ on each side, the fourth one shown by Bütschli probably belonging to the other side. In the ventro-lateral series of papillæ Bütschli's drawing does not show the one situated immediately post-anal but shows the two pre-anal ones which are found fairly close together in Tylopharynx.

8. *Cuticular Striations.* Bütschli mentions longitudinal striations on the cuticle of *A. fœtidus*. Such striations are uncommon on Aphelenchi but are very noticeable on Tylopharynx.

Aphelenchus nivalis Aurivillius, 1883.

Worms ascribed to this species occurred under some red-yellow alga, *Sphærella nivalis*, which came originally from the snow at Alkhornet, Spitzbergen. The algal material had become dry and was subsequently moistened with distilled water and the nematodes were found in this material under laboratory conditions. After a careful study of the original paper, the writer finds considerable difficulty in accepting this species as a member of the genus Aphelenchus, for the following reasons :—

1. *Size.* The measurements given reveal comparatively large, stout worms of both sexes, the females attaining a length of 2 mm. with a breadth of 0·1 mm., and the males an average of 1·47 mm. long by 0·07 mm. in width. These dimensions are much larger than those for any Aphelenchi.

2. *Female Gonad.* This is paired, opposed and reflexed with the posterior gonad rather shorter than the anterior one. This arrangement of the gonad is not found in true Aphelenchi.

3. *Anterior End.* The head is very distinctly set off from the body with a well-marked groove behind, giving it an appearance not met with in members of the genus Aphelenchus.

4. *Spicules.* Neither the description nor drawings of the spicules are sufficiently detailed to enable one to determine their shape and size. They are spoken of as longitudinally outstretched and the figures show two comparatively long bodies quite unlike the thorn-shaped structures characteristic of male Aphelenchi.

5. *Bristles and Papillæ.* These are described and figured as occurring on the cuticle chiefly towards the posterior end of both sexes. The drawings reveal structures which are very far from having the appearance of true setæ and papillæ but have much more the appearance presented by adherent rods of bacteria irregularly arranged in the case of the bristles and of small foreign bodies in the case of the so-called papillæ. The latter are very irregularly distributed in the caudal region and are quite unlike the true papillæ of Aphelenchi. Neither bristles nor papillæ can be taken, in the writer's opinion, to represent anything of morphological significance.

It is difficult to suggest to what genus *A. nivalis* should be assigned but the points given above are sufficient to show that it does not rightly belong to the genus Aphelenchus.

Alphabetical List of Aphelenchus Species.

A. aderholdi Schwartz, 1912, species inquirendæ, diseased roots of Lily-of-the-Valley, Germany.

A. agricola de Man, 1881 = *A. avenæ*, free-living about the roots of plants, Holland.

A. avenæ Bastian, 1865, type species, fully described (Goodey), 1927, p. 210. In decaying roots, bulbs, etc., cosmopolitan.

A. caprifici (Gasparrini, 1871) Cobb, 1927, within fig fruits on the fig-wasp *Blastophaga*, Algiers.

A. chamelocephalus Steiner, 1926, diseased pea-nut plants, South Africa.

A. coffeæ Noack, 1898, sp. inquirendæ, roots of coffee, Brazil.

A. coffeæ Zimmermann, 1898 = *A. parietinus*, East Indies.

A. demani n. sp., free-living from hop-roots, narcissus bulb, potato and grass, Holland and England.

A. dubius Steiner, 1914 = *Tylenchus robustus*, Switzerland.

A. dubius var. *peruviensis* Steiner, 1920 = *Tylenchus robustus*, Peru.

A. elegans Micoletzky, 1914 = *A. helophilus*, Austria.

A. erraticus Linstow, 1876 = *A. parietinus*, rectum of *Lacerta vivipara*.

A. fœtidus Bütschli, 1874 = *Tylopharynx striata*, cow-dung, Germany.

A. fragariæ Ritzema Bos, 1891, parasitic in cultivated strawberry plants.

A. gœldii Steiner, 1914, free-living, damp humus, Switzerland.

A. helophilus de Man. 1880, free-living, about grass roots and from grass blades, Holland and England.

A. littoralis Hofmänner, 1915 = *A. parietinus*, freshwater, Switzerland.

A. longicaudatus Cobb, 1893, free-living, about roots of banana, Fiji.

A. microlaimus Cobb, 1893 = *A. parietinus*, grass, Australia.

A. minor Cobb, 1893 = *A. parietinus*, about banana roots, Fiji.

A. modestus de Man, 1876 = *A. parietinus*, meadow soil, Holland.

A. mycogenes, Schwartz, 1912, sp. inquirendæ, culture of fungus, Germany.

A. naticochensis Steiner, 1920, freshwater, Lake Naticocha, Peru.

A. neglectus Rensch, 1924 = *Tylenchus pratensis*, roots of cereals and other plants, Germany. U.S.A.

A. nivalis Aurivillius, 1883, not an Aphelenchus, from alga taken from snow, Spitzbergen.

A. olesistus Ritzema Bos, 1893, parasitic in fern fronds, widespread.

A. olesistus var. *longicollis* Schwartz, 1911, galls on cultivated violets, Germany.

A. ormerodis Ritzema Bos, 1891 = *A. parietinus* or *fragariæ*, Strawberry plants, England.

A. (*Paraphelenchus*) *pseudoparietinus* Micoletzky, 1921, raised to generic rank (Goodey) 1927. The male tail is figured by Micoletzky (1925), Pl. 8, fig. 32.

A. parietinus Bastian, 1865, free-living, cosmopolitan, soil, freshwater and decaying organic matter of various kinds.

A. penardi Steiner, 1914 = *A. parietinus*, moss, Switzerland.

A. phyllophagus Stewart, 1921 = *A. ritzema-bosi*, parasitic in *Chrysanthemum indicum*, England.

A. pseudolesistus n. sp., decaying oak leaves and gall on *Chrysanthemum maximum*, England.

A. pyri Bastian, 1865 = *A. parietinus*, decaying pear leaves, England.

A. retusus Cobb, 1927, dead fly-pupa, U.S.A.

A. ribes (Taylor, 1917) Goodey, 1923, black currant buds, England.

A. richtersi Steiner, 1914 = *A. parietinus*, moss, Switzerland.

A. ritzema-bosi Schwartz, 1911, parasitic on *Chrysanthemum indicum*, widespread, Europe and U.S.A.

A. rivalis Bütschli, 1873 = *A. parietinus*, freshwater, Germany.

A. sp. Ditlevsen, 1912 = *A. helophilus*, Denmark.

A. striatus Steiner, 1914 = *A. parietinus*, damp humus, Switzerland.

A. subtenuis Cobb, 1926, narcissus bulbs, U.S.A.

A. tenuicaudatus de Man, 1895, rotting orchid and banana material, England and U.S.A.

A. villosus Bastian, 1865 = *A. parietinus*.

A. winchesi Goodey, 1927, pig-manure, England.

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Journal of Helminthology, Vol. VI, *No.* 3, *September*, 1928, *pp.* 161-164.

On a species of Onchocerca from the Ox in West Africa.

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The species of Onchocerca described in this paper was received some months ago from Mr. J. L. Stewart, *M.C.*, M.R.C.V.S., from Tamale, Gold Coast Colony. The cysts containing the parasites are found most commonly in the subcuticular connective tissue of the inter-costal muscles; but they are also found elsewhere, usually in a superficial position. Mr. Stewart in a letter accompanying the material, draws attention to their superficial resemblance to *Cysticercus bovis*.

A considerable number of males were dissected out from these nodules—sometimes two males could be found in a single nodule—but no complete females could be obtained.

The males are about 45 mm. long, with a maximum breadth of about 0·145 mm. The length of the female could not be determined, but fragments, apparently from the middle region of the body, had a diameter of about 0·35 mm. The head end is similar in both sexes, and like the posterior extremity is almost free from striations. The cuticle of the male is conspicuously striated, while in the female, the characteristic rugæ seen in *O. volvulus* are present. They have a width of about 20μ and are separated from each other by an interval of about 30μ.

The mouth opening is a simple pore with no vestibule. It is surrounded by four minute papillæ.

The œsophagus is elongated (about 0·7 to 0·8 mm.) and has an almost uniform diameter from the mouth opening to just in front of its junction with the intestine. At this point it is very slightly swollen (fig. A). The nerve ring surrounds the œsophagus at about the level of the anterior and second quarter and the excretory pore is just posterior to this.

The vulva opens at the level of the junction of œsophagus and intestine. The vagina consists of a long, muscular, backwardly directed tube which bifurcates to form the two parallel uteri.

The tail of the female is bluntly pointed and the anus opens about 0·2 mm. from the tip.

The ova are about 40μ long by 25μ wide and vary very much in shape. They hatch *in utero*.

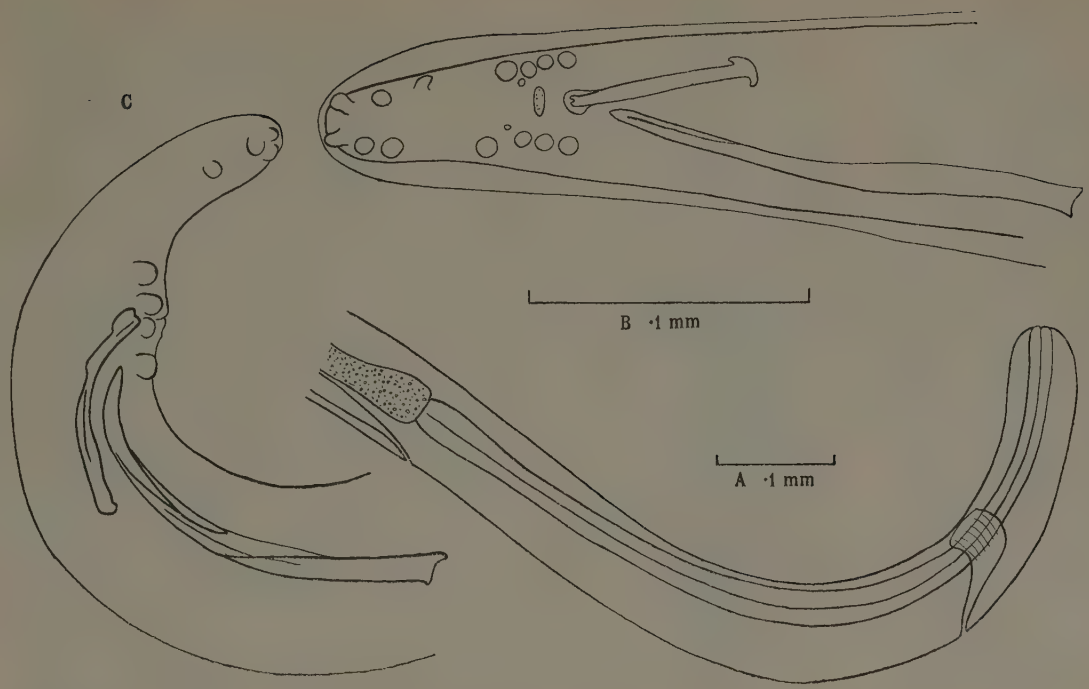
In the male the tail is spirally coiled and ends bluntly. The anogenital opening is about 0·075 mm. from the tip. Narrow caudal alæ are present. Typically (figs. B and C) there are four pairs of adanal papillæ, of which one pair is very small. This number varies considerably however, and there may be four on one side and three on the other : or even three on each side.

In addition there are three pairs of caudal papillæ which are arranged in a very haphazard fashion. No two specimens are alike. These papillæ are very small and sessile.

The spicules have the shape characteristic of the genus. The large spicule is 0·17 mm. long and is thickened proximally,and fluted distally. It has an opening in the middle of its length. The spicular sheath is transversely striated. The small spicule is curved, thickened proximally, and expanded and deepened distally. There is no accessory piece.

Morphologically, there does not appear to be any valid differences between this species and *O. volvulus* and *O. gibsoni*. The measurements are approximately the same. There are slight differences, however, in the arrangement and number of the papillæ in the male.

Leiper found four pairs of adanal in *O. gibsoni :* Fülleborn found three pairs of adanal and two pairs of caudal papillæ in *O. volvulus*, as also did Brumpt ; Prout however described four pairs of adanal papillæ in this species. As has been mentioned above, there is a considerable variation of these papillæ within the same species and too much reliance cannot be placed on their number and arrangement as a specific character.



Onchocerca sp. Fig. A.—Female, anterior end. Fig. B.—Tail of male, ventral view. Fig. C.—Tail of male, lateral view.

It is probable that *O. gibsoni* is a valid species as it is found only in cattle in Australia. On the other hand, *O. volvulus* and the present specimens have a coincident geographical distribution. It is impossible however, to say to which—if either—of these two species the present forms should be referred until a more detailed morphological and biological study of *O. volvulus* and *O. gibsoni* has been undertaken.

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On the habitat of *Ælurostrongylus abstrusus*, the lung worm of the Cat.

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Ælurostrongylus abstrusus, discovered by Mueller in the lungs of cats in Germany, has since been reported from various other countries in Europe. In many cases the diagnosis has been based on the recovery of the characteristic larva but occasionally a few adults have been found. These have generally been isolated by teasing up the lung substance in normal saline and then searching through the fluid by means of a low-powered objective or a dissecting microscope. The technique adopted by the writer was to cut thin slices of lung tissue, to compress these between two ordinary glass slides, and to examine them with an inch objective. The larvæ could be easily found in the expressed fluid and adults could be noticed as dark masses in the lung itself. When seen, the adults were removed by means of dissecting needles.

The exact habitat of the adults has however never been described. There are four possible localities in the lungs where parasitic worms can live: in the bronchi or bronchioles, in the alveoli, in the interstitial tissue, or finally, in the blood vessels of the lung.

During a study of the lesions provoked by this parasite in the lungs of the cat, one was struck by two peculiar facts. Firstly, adults were never seen in several hundreds of sections examined, although eggs and larvæ were plentiful. The adults were only found in teased specimens. (The sections were mainly prepared from marginal portions of the lung.) Secondly, the eggs in varying stages of development were evenly distributed throughout the lung and were not isolated in any particular area.

These facts suggested the possibility of a blood distribution of the eggs, and the blood stream as the normal habitat of the adults.

The infection was diagnosed by the presence of the characteristic larvæ in the fæces or in small portions of lung examined as described above. The vessels between the heart and the lung were then ligatured close to each of these organs, but without in any way interfering with them. The ligatured portion of the vessels was then removed, opened in saline and the contents shaken out. In several cases, fully adult male and female specimens of *Ælurostrongylus* were found in these vessels. The lung itself was then removed from the body, and still other adults were recovered in the larger branches of the pulmonary vessels.

The detailed examination of the host reactions to this parasite indicates that the adult parasites become mature in the pulmonary vessels, and the female deposits her large, thin-shelled eggs into the blood stream. They are mechanically carried to the capillaries of the lung where their progress is arrested and where the unsegmented ovum develops into the characteristic larva. When it is mature, the larvæ force their way through the thin-walled tissue into the alveoli, and in this way reach the exterior after traversing the bronchioles, bronchi, trachea and alimentary tract. Occasionally in very young animals and with very heavy infections, the eggs themselves rupture the capillaries and, escaping in an undeveloped condition into the alveoli, then continue their development.

While the present writer has never seen larvæ outside of the respiratory or alimentary tracts, Leuckart has described them from various parts of the body. Leuckart believed that he was dealing with the larvæ of *Ollulanus*, but in that he was undoubtedly mistaken : his infections were, unknown to him, mixed ones, of both the lung and the stomach forms. It is possible that these encysted larvæ were those of *Trichinella*, but their distribution does not lend support to this hypothesis, as they occurred in situations where *Trichinella* larvæ rarely are found. From the above discussion, it would seem more probable that they were larvæ which had—either as ova or larvæ—passed *through* the pulmonary capillary network and so been carried to the various organs by the blood stream. The encystment would be the natural reaction of the host tissues to any foreign body.

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Observations on Artificial Infestation of Sheep with *Fasciola hepatica* and on A Phase in the Development of the Parasite.

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By the close of the winter of 1925-26, the author's investigations into the value of certain drugs in the treatment and control of liver rot of sheep had reached a stage when it was felt that further advancement in certain important directions necessitated the employment of sheep subjects which had become infested with *Fasciola hepatica* to a known degree at a particular time. Naturally infested sheep were unsuitable, since observation had demonstrated a great variation—as regards both extent and type—in the infestation of sheep of the same flock.

It was decided to attempt artificially to infest suitable hosts. Preliminary experiments having indicated suitable general lines of procedure, the observations made during the winter seasons 1926-27 and 1927-28 are now placed on record.

Advantage was taken of this opportunity to study to some extent the development of *F. hepatica* within the host. So far as the writer is aware no accurate information regarding the time which elapses between the ingestion of cercarial cysts and the appearance of ova in the fæces of sheep is available. This point is of considerable practical importance and observations have been noted regarding it.

1926-27 Observations.

Subjects.—The sheep used in these experiments were wether lambs drawn from the mountain flock of the College Farm. These sheep are known to be very free from natural infestation. Throughout the experimental period they grazed the driest lowland fields available with a number of control sheep from the same flock.

Collection of Cercarial Cysts.—Many snails, *Limnæa truncatula*, were collected from known liver rot areas, placed in glass pots and observed to detect infestation. Cercariæ issuing from *Limnæa truncatula* and characterised by a single long tail, a peculiarly milky-white appearance, and the habit of rapid encystment were accepted as being those of the common liver fluke. Preliminary experiments, using guinea pigs, rabbits and sheep, assisted their identification. Wright (1) has described the cercaria here accepted as of *F. hepatica.* The writer is not aware of other cercariæ found in this province which possess these characters. It is possible, though extremely unlikely, that some cercarial cysts belonged to some other fluke not yet identified.

The cercariæ were allowed to encyst on the side of the glass vessel containing the snails. This they did readily ; most often at the general water level, frequently below it and occasionally in the water film forming the extreme edge of the meniscus. A very small proportion encysted free on the surface of the water.

Infestation of Experimental Animals.—The encysted cercariæ were carefully removed with a camel-hair brush and placed on the surface of pieces of damp lettuce leaf. The number was checked by examination with a hand lens. These pieces of leaf were then enclosed in hard gelatine capsules and administered to the sheep.

Collection and Examination of Fæces.—An examination was made of a sample of fæces obtained from the rectum of each sheep on the day previous to infestation and at weekly intervals thereafter. Prior to the commencement of the experiments none of the sheep were passing fluke ova.

Post-mortem Examination.—Each sheep was slaughtered fourteen weeks from the date of infestation. The liver was quickly removed and examined to detect signs of infestation—scar formation in the capsule, necrotic tracks, etc. A very careful search for flukes was made by open-

ing up the bile ducts throughout their length with fine pointed scissors and by squeezing the liver substance. Thereafter the liver was cut into thin slices. These were allowed to remain on the slab some two or three minutes before the final thorough search. The flukes recovered were placed in saline and fixed in Schaudinn's solution between glass plates, every endeavour being made to obtain a uniform pressure on them.

Observation No. 1.

The detail of this experiment is given in Table No. 1. No clinical difference distinguished the infested from the control sheep. All the flukes recovered from the experimental sheep were *F. hepatica*, but the stage of development reached varied markedly. Some appeared fully mature, the uterus being packed with characteristic brown-shelled

TABLE No. 1.

Sheep.	Number of cercarial cysts fed.	Number of ova found (per gram of natural fæces). Weeks after infestation.					Post-mortem Examination.
		at and before 10.	11.	12.	13.	14.	Number of flukes found.
B4	100	Nil	Nil	Nil	166	133	25
B5	100	Nil	Nil	Nil	33	133	17
B6	100	Nil	33	266	266	333	52
B12	100	Nil	33	100	300	233	27
B17	100	Nil	Nil	Nil	Nil	66	8
B18	100	Nil	Nil	Nil	Nil	33	13
Y7	100	Nil	Nil	33	66	166	43
B21 to B40	Not experimentally infested (Controls)	Nil	Nil	Nil	Nil	Nil	None
Y11		Nil	Nil	Nil	Nil	Nil	1
Y12		Nil	Nil	Nil	Nil	Nil	None
Y13		Nil	Nil	Nil	Nil	Nil	1
Y14		Nil	Nil	Nil	Nil	Nil	None

ova. In others only a proportion of the ova were fully formed while less mature individuals, in which the few eggs present were still in a grey shelled stage, were seen. A variation in the size of the flukes was obvious at the time of post-mortem and after fixation. Each of the parasites obtained from the control sheep was mature.

Observation No. 2.

Table No. 2. gives details of this experiment—the controls were the non-infested sheep of Observation No. 1. The flukes recovered varied rather less in size and development than those of the previous observation.

TABLE No. 2.

Sheep.	Number of cercarial cysts fed.	Number of ova found (per gram of natural fæces). Weeks after infestation.					Post-mortem Examination.
		at and before 10.	11.	12.	13.	14.	Number of flukes found.
R46	50	Nil	Nil	Nil	66	66	12
R47	25	Nil	Nil	Nil	Nil	33	12
R48	25	Nil	Nil	Nil	Nil	100	14
R49	25	Nil	Nil	Nil	Nil	Nil	19
R50	25	Nil	Nil	Nil	Nil	Nil	10

1927-28 Observations.

The subjects were drawn from the same flock and the routine followed was similar to that of the previous season. Very heavy rains caused some flooding of the lowland fields and rendered the pastures less "safe." The labour involved in fæcal examinations had to be reduced. Samples were taken on and after the tenth week from the date of infestation. The sheep in this series were slaughtered thirteen weeks after infestation.

Observation No. 3.

Details are given in Table No. 3. The variation in the stage of development and size exhibited by the flukes was more marked than in the two previous observations. This is probably accounted for by the fact that the sheep were killed one week earlier. In each case, while the majority of the flukes were definitely mature immature forms, even to individuals in which no ova could be detected, were present among the parasites recovered. Each of the five flukes obtained from the single control found to be infested was mature.

Observation No. 4.

The detail of this experiment is given in Table No. 4. Obvious variation was seen in the size and stage of development reached by the parasites recovered. Each of the eight flukes found in the control sheep was mature.

TABLE No. 3.

Sheep.	Number of cercarial cysts fed.	Number of ova found (per gram of natural fæces). Weeks after infestation. 10.	11.	12.	Post-mortem examination. Number of flukes found.
Y35	100	*	100	66	34
Y36	100	Nil	166	66	29
Y37	100	Nil	66	266	37
Y38	100	66	300	533	58
Y39	100	Nil	66	66	38
Y40	100	Nil	166	100	53
Y41	100	*	100	233	28
Y42	100	Nil	200	450	40
Y43	100	166	200	200	51
Y44	100	Nil	66	200	35
Y45	Not	Nil	Nil	Nil	None
Y46	experimentally	Nil	Nil	Nil	None
Y47	infested	†	Nil	66	5
Y48 to Y54	(Controls)	Nil	Nil	Nil	None

* Ova present in numbers too small to be estimated.
† No sample of fæces available.

TABLE No. 4.

Sheep.	Number of cercarial cysts fed.	Number of ova found (per gram of natural fæces). Weeks after infestation. 10.	11.	12.	Post-mortem examination. Number of flukes found.
B28	70	†	33	100	32
B29	70	33	†	33	38
B30	70	*	66	66	19
B31	70	*	100	33	41
B32	70	Nil	Nil	100	25
B33	70	Nil	†	*	28
B34	70	Nil	Nil	*	38
B35	70	Nil	33	133	18
B36	70	Nil	66	100	26
B37	70	Nil	100	33	34
B38		†	33	33	2
B39 to B43		Nil	Nil	Nil	None
B44	Not	Nil	33	100	5
B45	experimentally	†	†	Nil	None
B46	infested	Nil	33	*	1
B47	(Controls)	Nil	Nil	Nil	None

* Ova present in numbers too small to be estimated.
† No sample of fæces available.

Discussion.

It appears easily practicable to infest sheep artificially with *Fasciola hepatica*, and thus obtain subjects eminently suitable for the study of liver rot and of the value of therapeutic measures in its treatment and control.

The extent of infestation following the administration of a known number of cercarial cysts varies within quite wide limits. A number of sheep must therefore be employed for experimentation and as infested controls before conclusions can be drawn from any set of observations.

There may be many factors which influence the chance these minute forms have of reaching the intestine of a suitable host in an infective condition. Thereafter the larval fluke is exposed to many dangers before it reaches the liver and, finally, the bile ducts. The writer hopes to study the effect of drying, frost, etc., on the vitality of the cercarial cyst during the coming season. Some of the observations recorded suggest that a larger proportion of the cysts develop when the number given is small. Thus under natural conditions, when the host may pick up a small number of cysts almost daily, a proportionately heavier infestation may result.

That the rate of growth and development of flukes following the simultaneous ingestion of a number of cysts varies much, is of significance. It may result in flukes picked up at the same time becoming assailable by drugs at varying periods. These variations may possibly be accounted for by variations in the time spent by the larval flukes in wandering in search of the liver.

Sheep may commence to pass ova ten weeks after infestation, more commonly about the eleventh week or not even after fourteen weeks. This variation appears to confirm the observation that the rate of development of individual parasites is not constant, but may also be related to the extent of infestation and other factors. The observation is of considerable practical importance since it aids decision as to the times at which sheep should be treated in any effort to control the ravages of liver rot by the destruction of flukes as they mature.

Summary.

The successful artificial infestation of 32 sheep with *Fasciola hepatica* is recorded.

The collection and identification of the cercarial cysts and their administration to the sheep is detailed.

The experiments may be summarised as follows :—

Observation No. 1 (1926-27).

100 cercarial cysts — to each of 7 sheep.
52 liver flukes — maximum resulting infestation.
8 liver flukes — minimum resulting infestation.
26·4 liver flukes — average resulting infestation
1 liver fluke found in each of two of the 24 controls.

Observation No. 2 (1926-27).

50 cercarial cysts — to one sheep and 12 flukes recovered.
25 cercarial cysts — to each of four sheep.
19 liver flukes — maximum resulting infestation.
10 liver flukes — minimum resulting infestation.
13·75 liver flukes — average resulting infestation.
Controls : those of the previous experiment.

Observation No. 3 (1927-28).

100 cercarial cysts — to each of 10 sheep.
58 liver flukes — maximum resulting infestation.
28 liver flukes — minimum resulting infestation.
40·3 liver flukes — average resulting infestation.
Controls : 5 liver flukes recovered from one of the 10 controls.

Observation No. 4 (1927-28).

70 cercarial cysts — to each of 10 sheep.
41 liver flukes — maximum resulting infestation.
18 liver flukes — minimum resulting infestation.
29·9 liver flukes — average resulting infestation.
Controls : 3 of the 10 controls harboured flukes—2, 5 and 1 parasite respectively.

Following the administration of 2,550 cercarial cysts to thrirty-two sheep, 954 liver flukes were recovered on post-mortem examination—37·4 per cent. of the cysts developed. The smallest infestation produced was 8 per cent. (eight flukes from a sheep given 100 cercariæ) and

the heaviest 76 per cent. (nineteen flukes from a sheep given 25 cysts).

From forty-four sheep of the same general flock grazing throughout with the experimental animals fifteen flukes, 0·3 per head, were recovered. Five flukes were found in each of two, two in one and a single fluke in each of the other three controls found infested.

The parasites recovered from each experimental sheep were not uniform in size and the stage of development reached varied much.

Ova of the liver fluke were first found, in numbers sufficiently large to estimate, in the fæces of experimental sheep :—

in 3 cases 10 weeks after infestation,
in 16 cases 11 weeks after infestation,
in 2 cases 12 weeks after infestation,
in 3 cases 13 weeks after infestation, and
in 4 cases 14 weeks after infestation.

Eggs were only detected in the fæces of two sheep at their final, twelfth week, examination. Two sheep were not passing eggs in numbers sufficiently large to estimate even fourteen weeks after infestation.

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New Trematodes from the Newt *Triturus viridescens*.

By Fred J. Holl

(*Duke University.*)

The writer has found three apparently new species of trematodes in the newt, *Triturus viridescens* Rafinesque. The newts were collected near Durham and Lakeview, North Carolina, and a study is being made to determine if there is any seasonal periodicity in the occurrence of their parasites. It is of interest that one trematode, *Opisthodiscus americanus*, is the first species of its genus to be reported for North America. Only one species of *Brachycœlium* has been reported previously for North America and another, *B. trituri*, is described in this paper. A new species, *Gorgoderina intermedia*, is also described.

Opisthodiscus americanus, n. sp. (Figs. 1-4).

This fluke has a cornuate body. Near the anterior end it is circular in cross-sections, becomes ellipsoidal in the middle of the body, and again circular near the posterior sucker.

The specimen described measured 1·07 mm. in length; 0·357 mm. in width opposite the esophageal bulb and 0·663 mm. just anterior to the posterior sucker. The posterior sucker is large, about 0·748 mm. in diameter; surrounded by a rim 0·068 mm. wide; and faces postero-ventrally. In the centre of the posterior sucker is a plug, 0·153 mm. in diameter, the centre of which forms a secondary sucker. The oral sucker is large, 0·13 mm. long and 0·102 mm. wide.

Two pharyngeal pockets arise as diverticula from the posterio-dorsal wall of the oral sucker and extend dorsally. There is a common median wall between the two pockets (Fig. 3). The pharynx is narrow and 0·153 mm. long. It arises from the posterio-ventral region of the oral sucker and extends ventrally to the pharyngeal pockets, then curves dorsally behind them, to a median position. The pharynx becomes expanded posteriorly to form an esophageal bulb, 0·102 mm. long and 0·051 mm. wide. The intestinal rami arise posterior to the esophageal bulb and extend to within 0·16 mm. of the posterior sucker. The testes are slightly lobate ; equal in size (0·085 mm. by 0·119 mm.) ; and the right is slightly posterior to the left. The ovary is in the posterior region of the body, sinestral, directly behind the left testis, and measures 0·102 mm. by 0·119 mm. Mehlis' gland is to the right and dorsal to the ovary. Laurer's canal opens to the outside at the level of the ovary and arises from the oviduct near its entrance into Mehlis' gland. The follicular vitellaria are confined to the posterior third of the body, and lie just median to the intestinal rami. The uterus coils anteriorly from Mehlis' gland to the genital pore which is on the ventral side, just posterior to the bifurcation of the alimentary tract. The vasa deferentia lead into a cirrus sac which lies posterior to the genital pore. The cirrus sac and vagina unite to form a short duct which opens to the exterior through the genital pore.

The lateral tubes of the lymph system end on each side of the pharyngeal pockets and extend posteriorly. Each tube coils three times around an intestinal ramus.

The excretory bladder is located between the ovary and the posterior sucker. Two lateral tubes arise from it and extend into the anterior region of the body. The excretory pore is on the dorsal side just anterior to the posterior sucker.

Host : *Triturus viridescens* Rafinesque ; colon ; Type Locality : Durham, N.C.

Cohn (1904), in his studies on the sub-family Diplodiscinæ, divided the group into three genera :—(1) *Diplodiscus*, with two testes which are often fused in old specimens ; posterior sucker directed downward with a central opening ; excretory sytem with dark concretions ; and the genital pore near the oral sucker. (2) *Opisthodiscus*, with the posterior

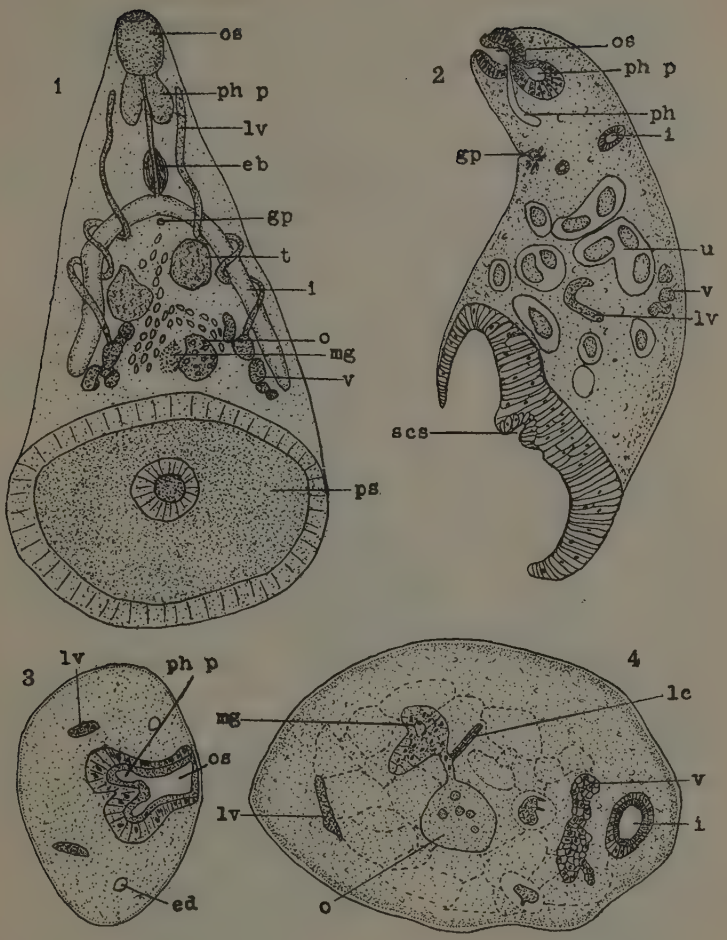

Fig. 1.—*Opisthodiscus americanus*, entire specimen flattened. × 105.

Fig. 2.—*Opisthodiscus americanus*, sagittal section through genital pore. × 105.

Fig. 3.—*Opisthodiscus americanus*, cross section of body through oral sucker and pharyngeal pockets. × 175.

Fig. 4.—*Opisthodiscus americanus*, cross section of body through ovary and Mehlis' gland. × 175.

sucker extending backward and including a prominent central sucking organ within it ; excretory system without dark concretions ; and genital pore near the oral sucker. (3) *Catadiscus*, with only one testis ; genital pore in middle of body ; esophagus long ; and excretory canal with dark concretions.

Stafford (1905) described *Diplodiscus temperatus* from frogs, *R. pipiens* Schreber and *R. catesbiana* (Shaw), collected in Canada. Chandler (1923) added a fourth genus to include *Megalodiscus americanus* from *Amphiuma means* Garden. Millzner (1924) added *M. ranophilius* from *Rana pipiens* to the genus created by Chandler. Chapin (1926) believed *M. ranophelia* Millzner to be the same as *Diplodiscus temperatus*. Cort (1926) places Chandler's species in the genus *Diplodiscus*. The writer has not examined any species of *Megalodiscus*, but believes that future work will show that there are a number of species, belonging to this group, in North America.

GORGODERINA INTERMEDIA, n. sp. (Figs. 5 and 6).

Specimens of *G. intermedia* containing eggs were found to range from 1·44 mm. to 2·76 mm. in length. The one described measured 2·01 mm. and had a width in the posterior region of 0·425 mm. ; width just posterior to oral sucker, 0·221 mm. The body posterior to the acetabulum is cylindrical ; the thickness varies from one half to three-fifths of the width. The body is flattened in the region of the acetabulum.

The oral sucker is 0·238 mm. in diameter ; acetabulum, 0·391 mm. The ratio between the oral sucker and acetabulum was studied in eight specimens, and was found to vary from 1 : 1·56 to 1 : 1·87 with an average of 1 : 1·7. In the type specimen the ratio was 1 : 1·84. The esophagus is 0·153 mm. long and the intestinal rami extend into the

EXPLANATION OF PLATES.

ac acetabulum
cs cirrus sac
e esophagus
eb esophageal bulb
ed excretory duct
lv lymph vessel
mg Mehlis' gland
o ovary
od oviduct
os oral sucker
ph pharynx
ps posterior sucker
ph p pharyngeal pockets
sc s secondary posterior sucker
t testis
u uterus
v vitellaria
i intestinal rami
l.c. Laurer's canal
vd vitelline duct

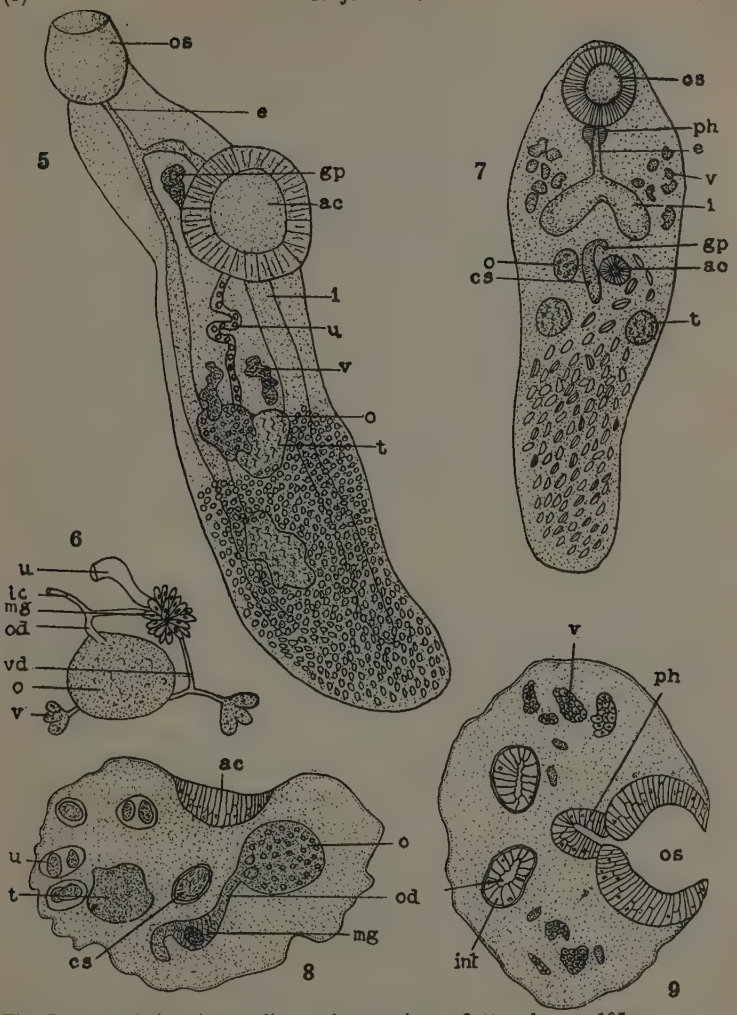

Fig. 5.—*Gorgoderina intermedia*, entire specimen flattened. × 105.

Fig. 6.—*Gorgoderina intermedia*, reconstruction of female genital apparatus from in toto and sections. × 105.

Fig. 7.—*Brachycœlium trituri*, entire specimen flattened. × 105.

Fig. 8.—*Brachycœlium trituri*, cross section through acetabulum and ovary. × 175.

Fig. 9.—*Brachycœlium trituri*, cross section through the oral sucker. × 175.

posterior fifth of the body. The rami vary in diameter, being widest in the region of the gonads.

The ovary is on the right side posterior to the acetabulum and measures 0·167 mm. by 0·114 mm. The oviduct arises on the dorsal side of the ovary, and about midway between the origin of the oviduct and Mehlis' gland arises Laurer's canal. The vitellaria are arranged into two compact masses, one on each side of the body, and are just anterior to the ovary. The vitelline ducts of each side unite to form a common canal lateral to the ovary. The slightly lobate anterior testis, 0·16 mm. by 0·106 mm., is immediately ventral and posterior to the ovary on the median line. The elongated and slightly lobate posterior testis, 0·106 mm. by 0·078 mm., is a little distance behind the anterior testis on the right side of the body. The genital pore is located between the bifurcation of the alimentary tract and the acetabulum. The male ducts form a large cirrus sac just before reaching the genital pore. The uterus is closely coiled in the posterior third of the body and is filled with many eggs. Several distinct coils of the uterus can be seen anterior to the vitellaria.

Host: *Triturus viridescens:* urinary bladder; Type Locality: Lakeview, N.C.

Cort (1912) monographed the bladder flukes of frogs, concluded that Stafford's *Gorgoderina simplex* and *G. opaca* were not distinct species, and adopted the former name. In the present species, *G. intermedia*, the oral sucker has a ratio to the acetabulum which is intermediate between that of *G. simplex* Stafford, and *G. attenuata* Stafford. *G. simplex* has an acetabulum which is less than 1·5 times the diameter of the oral sucker, while in *G. attenuata* the acetabulum is more than twice the diameter of oral sucker.

Brachycœlium trituri, n. sp. (Figs. 7-9).

Brachycœlium trituri was found in the intestine of *Triturus viridescens*. The length of the worm described is 1·4 mm. The width opposite the acetabulum is 0·357 mm., and opposite the bifurcation of the alimentary tract is 0·442 mm. At the anterior end is the large oral sucker which has a diameter of 0·1672 mm. The small acetabulum near the middle of the body has a diameter of 0·0836 mm. The pharynx is 0·0418 mm. long and 0·038 mm. in width. A narrow esophagus 0·076 mm. long divides into two short saccate intestinal rami, which are lined internally by large cells. The rami terminate anterior to the genital pore, which is

anterior to the acetabulum. A large pyriform cirrus sac, 0·1336 mm. long by 0·0608 mm. wide, lies to the right of the acetabulum. The oval ovary, 0·0532 mm. by 0·0912 mm., is located to the right of the acetabulum and is separated from it by the cirrus sac. The oviduct arises from the medio-lateral angle of the ovary and extends medially and dorsally towards the shell gland. The uterus is coiled in the posterior third of the body and contains about 125 thick shelled eggs. The follicular vitellaria are lateral to the esophagus and intestinal rami. The globular testes are situated on each side of the body just posterior to the cirrus sac and are about equal in size. The left testis is 0·076 mm. by 0·095 mm. and the right 0·0836 mm. by 0·076 mm.

Host: *Triturus viridescens* Rafinesque; intestine; Type Locality: Lakeview, N.C.

Brachycœlium hospitale was described by Stafford (1900) under the name *Distomum hospitale*, and later (1902) he referred to it as *Brachycœlium hospitale*. The new species, *B. trituri*, is a parasite in the same species of salamander from which Stafford reported *B. hospitale*. The newts from which the writer collected *B. trituri* are placed in the species *Triturus viridescens* Rafinesque, but the red spots on their sides tended to form in a line, which is characteristic of *T. viridescens dorsalis* (Harlan). All newts collected at Lakeview, N.C., showed this intermediate condition between *T. viridescens* and *T. viridescens dorsalis*. *B. trituri* has been compared with a specimen of *B. hospitale*, kindly loaned the writer by Dr. H. W. Stunkard. The intestinal rami are larger in the writer's species. The arrangement of the vitellaria is different in the two species: in *B. hospitale* they extend posterior to the termination of the alimentary tract and are median to the rami; in twelve specimens of the new species the vitellaria were never posterior to the alimentary tract and never median to the rami. In a search of the literature, one other closely related species, *Margeana californiensis*, Cort (1919), is found to have been reported from North American amphibian.

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On the Infective Larva of *Ostertagia circumcincta* (Stadelmann, 1894), a Stomach Parasite of Sheep.

By D. O. Morgan, M.Sc., Ph.D.

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Introduction.

Although the morphology of the adult stage of the majority of the helminthic parasites of domestic stock is now well known there still remains a considerable amount of work to be done on the larval stages of these worms. Some of the more recent researches on the infective stage of human and other hookworms have shown that a close study of the morphology reveals appreciable differences between the larval stages of closely allied species and that the differences can be used as diagnostic characters.

In this communication an account is given both of the morphology and the biology of the third stage larva of *Ostertagia circumcincta* (Stadelmann, 1894). This is a very common parasite of the abomasum of sheep and other animals and has a wide distribution. In association with *Hæmonchus contortus* it undoubtedly plays an important part in the incidence of parasitic gastritis in these hosts.

Technique.

Since it is rare to find a sheep with no other parasites than *O. circumcincta* it would be difficult to obtain a pure culture of the larval stages of this worm by making cultures from the fæces of a heavily infected host.

It was therefore necessary to obtain eggs by collecting a large number of females from the abomasum. In this way one eliminated the chances of contamination with the eggs of other parasites, with the possible exception of *Ostertagia trifurcata*. Up to the present the differences between the females of these two species of *Ostertagia* inhabiting the abomasum of sheep is not sufficiently clear and in any case an attempt to separate them would entail a very close scrutiny of each individual under fairly high magnification. This would make the task of obtaining a sufficient number of *O. circumcincta* females for cultures, without any possible chance of contamination, too long and tedious. Further, since *O. trifurcata* is a much rarer species than *O. circumcincta* and is only present as a rule in very small numbers it was decided to disregard this small chance of contamination.

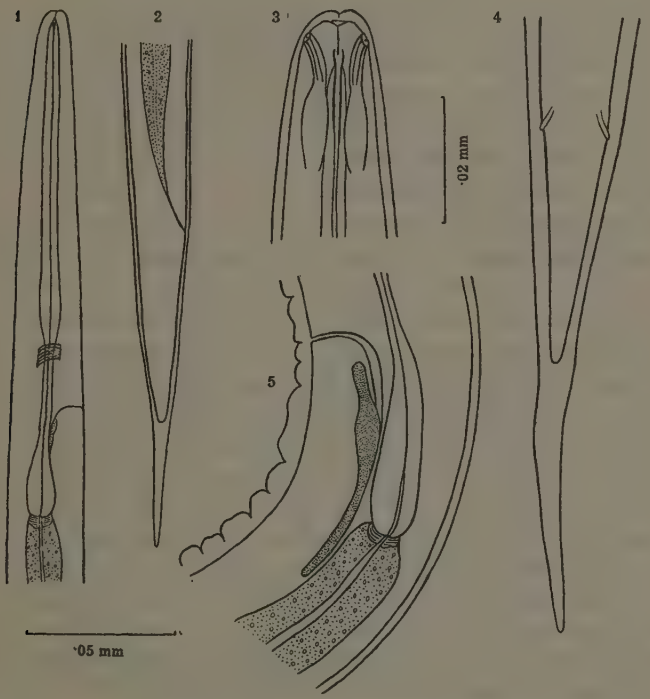
The first attempts to culture the larvæ in water showed that hatching took place readily, but no further development to the infective stage was obtained. Subsequently, cultures were made in the usual way with sterilised sheep's droppings mixed with animal charcoal. The female worms were either placed in this mixture whole, or cut up into small pieces. Either of these methods gave quite good results and third stage larvæ were obtained within a week at laboratory temperatures in the summer months. Since the larvæ climb quite readily on to the filter paper lining the lid of the Petri dish they were easily recovered by flooding this with water.

For examination under the microscope, the larvæ were placed in a small quantity of water on a slide and were killed by holding the slide for a short time over a flame. Care was taken not to apply too much heat and the best results were obtained when the larvæ were not quite dead but remained sufficiently dormant that their examination was not interfered with.

Morphology of the Infective Larva.

The infective larva of *O. circumcincta* measures 0·83 mm. in length with a greatest breadth of 0·03 mm. including the sheath. The head end of the worm is bluntly rounded and has a width of 0·01 mm. It increases gradually in width up to the region of the anterior part of the intestine and maintains this width until it reaches a point a little

in front of the anus. From this latter position towards the posterior end it narrows gradually, ending almost in a point at the tip of the tail. The width at the anus is 0·02 mm. including that of the sheath.



Ensheathed Larva of *Ostertagia circumcincta*.

Fig. 1.—Anterior region showing shape of œsophagus, position of nerve ring and excretory pore.

Fig. 2.—Posterior end showing position of anus.

Fig. 3.—Head end under high magnification in dorsal view showing amphids and arrangement of buccal region.

Fig. 4.—Posterior end under high magnification in dorsal view showing the shape of the tail and position of the lateral caudal papillæ.

Fig. 5.—Excretory apparatus under high magnification in lateral view.

The horizontal scale applies to Figs. 1 and 2 ; the vertical one to Figs. 3, 4 and 5.

The cuticle of the sheath is marked with fine transverse markings, the distance between them being approximately 0·0017 mm.

The mouth appears to be surrounded by three minute lips which have the usual arrangement of four papillæ. It opens into a short, cylindrical and narrow duct which soon widens out into a vase-shaped oral cavity having three cuticular thickenings. These thickenings are slightly curved or clasp-like and are prolongations of the parallel rods which mark the thickened portions of the wall of the lumen in the anterior part of the œsophagus. Two of these rods are almost of equal thickness but differ slightly in length. The third is represented by a very narrow cuticularised line.

The amphids are fairly distinct and communicate with the exterior a short distance behind the anterior end. The aperture of each amphid is fairly wide and leads into an amphidial pouch with thick walls. This widens out into a posterior region which is marked with nerve fibres.

The œsophagus is long and narrow and has two swellings or bulbs. The anterior bulb is quite rudimentary and could hardly be recognised as such but for the narrowing of the œsophagus immediately behind it. This bulb divides the œsophagus into two regions having a length ratio of 3 : 2. It is seen, therefore, to be placed more posteriorly than in the third stage larva of *Necator americanus* where the œsophagus is divided into two equal portions by this bulb (Schuurmans Stekhoven, 1926). Immediately behind the anterior bulb the œsophagus is crossed by the nerve ring. The posterior bulb is wider than the anterior one and is connected with the intestine by a sphincter. The granular regions which mark the position of the œsophageal (salivary) glands were observed in the posterior bulb but the exact limits of these glands could not be made out with any certainty.

The excretory pore is situated at a point 0·15 mm. from the anterior end. It leads into a narrow duct which widens out into the excretory ampulla. This latter organ, even when filled with the excretory contents, does not become vase-shaped, and the walls even under expansion remain almost parallel. The excretory cells appear to be H-shaped with short and fairly wide anterior horns. The middle region widens out but becomes narrower again towards the posterior end. This latter part, however, could not be seen very clearly.

The tail end of the larval sheath is moderately long and almost pointed with a slight kink immediately behind the posterior limits of the third stage larva. The tail of the latter is much wider than that of the second stage and is rounded at the tip. It has a length of 0·065 mm. and the sheath projects beyond this for a distance of 0·045 mm. A pair of minute lateral post-anal papillæ are situated about 0·04 mm. from the posterior end of the second stage larva.

The genital primordium is quite prominent and is situated about 0·45 mm. from the anterior end of the worm.

The recent publications of Schuurmans Stekhoven on the larval stages of hookworms give an excellent account of the minute structure of these worms, and it is now possible to make some comparisons between these hookworm larvæ and that of the Trichostrongyle described in this paper.

The organs of the head region in the third stage larva of *O. circumcincta* show a close similarity to those found in *Ancylostoma duodenale*. It is true that the lips in *O. circumcincta* are not very pronounced and also that the constriction immediately behind this region, which is a marked feature of *A. duodenale*, was not observed. The structure of the oral cavity and the anterior end of the œsophagus, however, appear to be very similar to the arrangement found in *A. duodenale*. Again, the amphids are on the same general plan as those in both *A. duodenale* and *A. caninum* with the exception that the constriction which separates the amphidial pouch and the posterior region of the amphid was not observed in *O. circumcincta*. This may have been due to the fact that the amphidial pouch is narrower and has parallel walls in the latter species and the constriction, if it exists, would therefore not be so pronounced.

The shape of the œsophagus and particularly that of the anterior bulb in *O. circumcincta* is like that of *Necator americanus*: somewhat less pronounced, perhaps, but not showing the sharp constriction immediately behind it as one finds in *A. duodenale* and *A. caninum*.

The excretory system appears to be on the same general plan as that found in *A. duodenale* and *A. caninum*.

As already stated the œsophageal glands were not made out in sufficient detail for comparison with those found in other larvæ.

Biology of the Infective Larva.

Effect of Heat.—The reactions of the third stage larva to heat were tested by means of an electrically heated stage placed on the microscope. On this stage a slide was placed and a drop of water containing larvæ in suspension was added. The movements of the larvæ were then observed at different temperatures up to 50° C. This was repeated four times with the following results.

Between 22-25° C. the larvæ were quite active, but became gradually less so as the temperature rose to 30° C. From 30-35° C. the movement was still less, while between 35° C. and 40° C. the majority coiled up and at the latter temperature almost all were still. Up to 45° C. some straightened out a little and showed slight jerky movements. None revived, however, on cooling after about half an hour's exposure to this temperature, except in the case of one larva, which showed slight movement at first, but later became quite still.

This result shows that these larvæ are most active at about a temperature of 25° C. and that they have a tendency to become gradually more lethargic as the temperature rises above this point. They do not revive after exposure to a temperature of 45° C. and over.

The reactions to very low temperatures have not been observed, but the larvæ were seen to be very sluggish at 18° C. and increased in activity as the temperature rose up to 25° C.

The reaction of the third stage larva of *O. circumcincta* to heat differs from that observed in the larvæ of some of the other species of sheep parasites. Cameron (1923) found in the case of *Monodontus trigonocephalus* that the greatest activity was exhibited by the larvæ between the temperatures of 35° C. and 40° C. Veglia (1916) obtained somewhat similar results in his experiments on the larvæ of *Hæmonchus contortus*. In the case of *Nematodirus filicollis*, however, Cameron (1923) has shown that the optimum temperature is at 23° C. and that the larvæ become quiescent at temperatures above 25° C. Goodey (1922) also found that the temperature for greatest activity in the larvæ of *Graphidium strigosum* and *Trichostrongylus retortæformis* is between 22° C. and 25° C. It will be seen, therefore, that the larvæ of *O. circumcincta* are like the larvæ of these latter species in their reactions to heat.

This high activity at about 23° C. with the subsequent inactivity as the temperature increases is somewhat remarkable when one considers the temperature at which these larvæ find themselves when they reach their host—a temperature in the region of 38° C. It seems probable as Goodey (1922) points out that the larvæ after ingestion by their host soon become accustomed to the new temperature, and that contact with the stomach wall is required in order to stimulate greater activity for moulting and further growth.

Desiccation.—Observations on the resistence of the larvæ to desiccation showed that they could withstand dry conditions for a considerable time. In this respect they resemble the larvæ of *N. filicollis* (*vide* Boulenger, 1915) and also *G. strigosum* and *T. retortæformis* (*vide* Goodey, 1922). Larvæ were placed on a slide and the water in which they were suspended was allowed to evaporate ; the slide was left in this condition overnight. In the morning water was added and the larvæ revived in a few minutes. When larvæ, however, were placed outside in direct sunlight for about two hours it was found that they did not revive later, on the addition of water. One has to consider in this latter experiment the effect of a fairly high temperature in addition to desiccation ; the temperature, at the time, being in the neighbourhood of 36° C. Also the exposure of the larvæ to direct sunlight may have had some effect in this instance. Exposure to direct sunlight did not kill the larvæ as long as they remained in a moist condition ; they became quite inactive it is true, but soon revived when the temperature was lowered. In the open field the larvæ would frequently be exposed to desiccation at a fairly high temperature during the summer months. The shady side of grass, however, would afford some protection against these conditions for so small an organism.

Thermotropism.—When a hot needle was brought to touch the under surface of a glass slide on which a suspension of larvæ in water had been placed the larvæ moved away from the source of heat. This experiment was repeated several times with the same result, and is in accord with the observations made in the experiments with the hot stage. It can, therefore, be said that the larvæ of *O. circumcincta* are negatively thermotropic at least for temperatures higher than 25° C.

Method of Infection.—An experiment was carried out to determine whether the third stage larvæ were able to infect their host through the skin ; the technique being that described by Goodey (1922).

A piece of skin from which the hair had been shaved off was cut out from the abdomen of a young mouse. This was stretched out on a cork ring with the external side upwards and then floated on saline which had been raised to a temperature of 37–40° C. A drop of water containing larvæ was placed on the skin and their movements watched under a binocular microscope.

The larvæ were not very active, in fact the majority soon coiled up and became quiescent. At intervals one or two would become fairly active with lashing movements in a horizontal plane, but without any downward attempt to penetrate the skin as one finds in the case of hook-worm larvæ. One may conclude, therefore, that the mode of infection in *O. circumcincta* is by the mouth.

Using the same technique as described above, a few larvæ were placed on the abomasum of a sheep. None of the larvæ showed any attempt to penetrate the mucous membrane and none of them became ex-sheathed. Cameron (1923) in his experiments on the larvæ of *M. trigonocephalus* found that the larvæ cast off their sheath in contact with the abomasum of sheep, but that none of them penetrated the mucous membrane. In the case of this latter species, however, the optimum temperature of the larvæ coincides with that of its final host, whereas in *O. circumcincta* it is much lower and the larvæ are far too inactive at the higher temperature to cast off their sheath.

Effect of Aniline Dyes.—The result of experiments by various workers on the effect of aniline dyes has shown that larvæ which are skin-penetrators become exsheathed in the presence of Fuchsin and other dyes and that only the sheath becomes stained. Non-skin-penetrators, however, do not exsheath but absorb the dyes very readily and are quickly killed.

The writer's observations on the larvæ of *O. circumcincta* do not bear out these conclusions. Although these larvæ are non-skin-penetrators they do not take up the stain very readily and are not killed by it. Further, it was found that the sheath alone took up the stain as in the case of skin-penetrators, and also that none of the larvæ became exsheathed although they showed considerable activity.

Phototropism.—Although repeated experiments were made, no definite indications were obtained of movements either towards or away from the source of light. It was observed that the larvæ became inactive when kept in a dark chamber and that they greatly increased their activity in a very short time on exposure to light. A narrow beam of light passing into a chamber containing larvæ in a glass well did not appear to stimulate them to activity. Neither did they become active when the whole undersurface of the dish was exposed to light on the stage of a microscope while the remainder was covered. Great activity was observed only when the dish containing the larvæ was fully exposed to light, and at the optimum temperature.

Longevity.—Complete experiments on the longevity of the larvæ have not been carried out, but up to the present it can be said that they are capable of living in water for at least three months. The duration of life under field conditions has also not been ascertained but it seems probable that the varying conditions met with would tend to stimulate greater activity than would be the case in the laboratory. This would hasten starvation and exhaustion.

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Observations on *Tylenchus musicola* Cobb, 1919, from diseased Banana Roots.

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In March, 1928, Mr. S. F. Ashby of the Imperial Bureau of Mycology, sent the writer some portions of diseased banana roots for examination and determination of the nematodes occurring therein. The material had come from plants growing in the Palm House at the Royal Botanic Gardens, Kew, where bananas of several species had been growing for some years in the same soil. Information was later sent that the plants had been in failing health for the last two or three years and that this year the symptoms of disease had become acute. These symptoms were said to be quite similar to those described by Nowell (1919) in the case of diseased " bluggoe " banana in Grenada with which *Tylenchus musicola* was found to be associated.

The material arrived in a fresh condition and was mainly of a dull blackish brown colour and comparatively soft. A small portion of it was teased up in water when specimens of *Tylenchus musicola* and of one or two other kinds of nematodes were obtained from the soft cortical tissues. The bulk of it was cut into small pieces and extracted by the Baermann method with cold water and by this means numbers of *T. musicola* were obtained. They were much more numerous than any other kind of nematode obtained from the material.

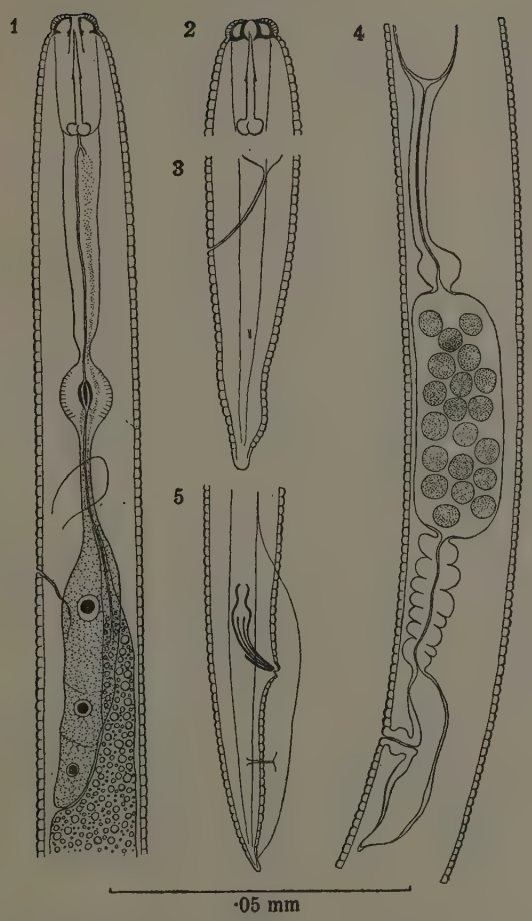
Tylenchus musicola has been recorded twice previously ; first by Cobb (1919) from roots of the " bluggoe " banana and recently by Steiner (1927) from vine roots. Cobb's material was preserved in 70 per cent. alcohol and Steiner's reached him in a dried condition, the worms being obtained by soaking in water. The writer has been fortunate in obtaining living worms from fresh material and the detailed structure of these has been studied in the living condition and immediately after killing by heat. Many of them were fixed by Ditlevsen's mixture and finally mounted in weak glycerine. In the main, the writer's observations confirm those of Cobb's original description, whilst they throw additional light on certain details which have not been fully made out by previous investigators.

Morphology.

Principal measurements.—Length, female, 0·64 mm. to 0·68 mm ; male, 0·55 mm. to 0·57 mm. Width, female, 0·02 mm. to 0·025 mm ; male, 0·017 mm. to 0·02 mm. ; stylet, 0·017 mm. ; anterior end to posterior side of œsophageal bulb, 0·07 mm. to 0·073 mm. ; anterior end to posterior end of salivary glands, 0·135 mm. to 0·14 mm. ; anterior end to vulva, 0·46 mm. to 0·5 mm. ; anus to tip of tail, female and male, 0·035 mm. ; spicules, 0·015 mm.

The cuticle is transversely striated with coarse striæ which, however, do not cross the rather prominent lateral fields. The body tapers slightly anteriorly and posteriorly and is comparatively stout in proportion to the length in both sexes. The head which also carries transverse striæ, is rather flat and is limited behind by a shallow encircling groove. The accompanying illustrations show that it is supported by a strongly cuticularised framework having the appearance shown in fig. 2 when seen in surface view. From this it seems clear that it is formed from six lips fused together. When viewed in optical section (see fig. 1) it can be seen that the head framework forms an almost cylindrical buccal cavity the walls of which have small lateral thickenings immediately inside the oral opening and at the level of the posterior limit of the head. Following this they are continued backward as somewhat fainter lines to a distance practically equal to the depth of the head.

The stylet is large and is composed of an anterior conical part about equal in length to the cylindrical posterior part on to which it fits. The basal swellings are large and prominent. Both Cobb and Steiner speak



Tylenchus musicola Cobb, 1919.

Fig. 1.—Anterior end of a worm drawn in optical section. Fig. 2.—Surface view of head region. Fig. 3.—Female tail. Fig. 4.—Portion of female gonad. Fig. 5.—Male tail. All drawings made under oil-immersion to the same scale of magnification.

of the somewhat "ghostly" appearance of the second part of the stylet and the swellings but this is probably because they only examined preserved specimens; in the fresh state the whole stylet is a remarkably distinct and refractive structure. It is surrounded by a clear zone probably composed of muscles attached to its base. The outlines of this zone pass into those of the anterior part of the œsophagus. The latter gradually decreases in diameter towards the muscular bulb immediately anterior to which it constricts to a narrow neck. This constriction was found to be a constant feature in all the specimens examined. The bulb is rather small and is succeeded by a slender part crossed by the nerve ring posterior to which the œsophagus gives off the salivary glands and gradually merges into the intestine. The salivary glands were not clearly made out by Cobb and Steiner. They are three in number, lying together in a compact mass, but the faint indications of the separate cells can be distinguished under the oil-immersion. The anterior cell is rather larger than the other two and in each a distinct nucleus can be seen. As fig. 1 shows the glands are situated ventro-laterally in the body with the posterior end close to the ventral side of the body. It has been found difficult to trace the course of the outlets from the glands but apparently there are two openings into the lumen of the œsophagus at the posterior end of the crescentic lining of the muscular bulb and a strand of granular material passes through the bulb, along the dorsal side of the anterior part of the œsophagus and opens by a short duct close to the base of the stylet. The excretory pore is situated a short distance posterior to the level of the nerve ring. The beginning of the intestine is overlaid by the salivary glands. It is quite narrow at the level of the excretory pore, but widens gradually until the posterior end of the glands is reached when it expands and occupies the width of the body. Its walls contain large numbers of fatty reserve food globules of various sizes.

Female characters.—The vulva, situated at about two-thirds of the body length from the anterior end has fairly prominent lips and the vagina which has thickened walls leads inwards at right angles to the body surface. It opens into a rather shallow uterine cavity, the walls of which are refractive in appearance. Posteriorly there is a short uterine sac about as long as the body is wide at this point. Anteriorly the cavity is a little shorter than the posterior one. It narrows down and leads into a muscular

portion with convoluted walls. This then expands into a well-defined, almost cylindrical receptaculum seminis which in most of the examples examined was filled with spherical sperms. In front of this there is another fairly thick walled oviduct which leads to the ovary. The latter is rather short and does not extend more than about half the distance from the vulva to the salivary glands. None of the worms examined showed ripe ova.

The shape of the female tail is an important character in that it differentiates this species from another root infesting member of genus, *Tylenchus pratensis* in which there is little tapering behind the anus and the tip is broad and bluntly rounded. Cobb has shown that in *T. musicola* the tip of the tail is rather variable in shape. In the specimens examined by the writer the majority had a shape like that shown in fig. 3. There is a good deal of tapering behind the anus and just anterior to the tip the dorsal side swells outward with a slighter corresponding inward curve on the ventral side the two sides then uniting to form a rather stout peg-like tip. On either side of the tail at about half-way between the level of the anus and the tip there is a fine lateral papilla arising from the lateral line and proceeding to a small pit in the cuticle.

Male characters.—The male tail tapers considerably behind the anus to a fairly sharp point. On each side of the body there is a caudal ala which arises in the region of the ventral margin of the lateral field some distance anterior to the forward end of the spicules and joins the body just in front of the tip of the tail. There is a single pair of caudal papillæ or bursal ribs situated about halfway between the anus and the end of the tail which arise, one on either side, from the lateral line and run outwards to about half the depth of each wing where the point lies in a shallow depression. The spicules are paired and each is shaped as in fig. 5.

The small slightly swollen head is followed by a constriction after which the spicule expands and attains its greatest width and then gradually tapers to a narrow point. There is a simple gubernaculum which is about one-third the length of the spicules.

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Journal of Helminthology, Vol. VI, *No.* 4, *December,* 1928, *pp.* 199-204.

A New Nematode Species of the Genus *Viannaia* from the Mole (*Talpa europæa*).

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Introduction.

A few specimens of the Trichostrongyle, described in this paper, were obtained from the intestine of moles caught on the experimental farm of the Institute of Agricultural Parasitology, St. Albans.

An examination of the parasites showed that they were distinctly different from the species of the family *Trichostrongylidæ* already described from this host, viz.: *Viannaia linstowi* (v. Linst., 1882) Travassos, 1921. The differences between the two species will be referred to later in this publication.

The writer, so far, has made no observations on the distribution of the parasite in this country. It occurred quite frequently in the moles examined from the above farm, but only a few specimens were found in each host.

Morphology.

The specimens were fixed in hot glycerine alcohol which was afterwards allowed to evaporate, and the material was then mounted in this medium. The worms were somewhat distorted and considerable difficulty was experienced in making out the details of some of the structures. This was particularly so in the case of the female genitalia.

The specimens were slightly coiled but not in a spiral as one finds in some members of this group of nematodes. Both the male and the female have a cuticle which is much inflated throughout the length of the body; it is also marked with a number of longitudinal ridges with fine transverse striations. These striations appear to be confined to the top of the ridges and do not extend into the furrows between them. The cuticle at the head end shows a cylindrical inflation which terminates in a groove situated about 0·04 mm. from the anterior end; it has well marked transverse striations. At a distance of about 0·15 mm. from the head end there is a deep pit on the mid ventral line which marks the position of the excretory pore. The mouth is surrounded by six papillæ and opens direct into the œsophagus. The latter widens gradually towards its posterior extremity where it reaches a width of 0·025 mm. Its length is 0·3 mm.

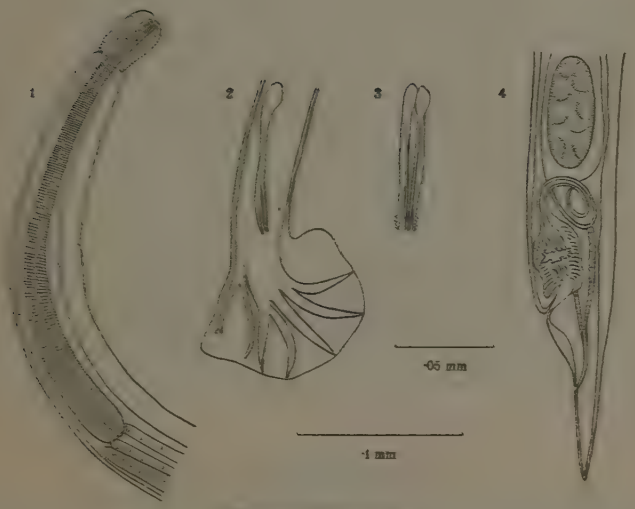
Female. The average length of the females examined is 2·2 mm. ranging from 1·8 mm. to 2·4 mm., and the greatest breadth 0·06 mm. The anterior end measures 0·025 mm. in width or 0·015 mm. excluding the cuticular dilation. The width then increases as far as the posterior end of the œsophagus and remains fairly uniform up to the region of the anus. The tail is fairly long and pointed and measures 0·06 mm. in length.

The vulva is situated about 0·05 mm. in front of the anus and opens into a short vagina which passes into an ovejector apparatus. This latter consists of a highly muscular region similar to that found in other members of the *Heligmosomina* and may be compared with the *pars ejectrix* of Trichostrongyles possessing a double uterus. Owing to considerable contraction in the worms the exact limits of this region of the ovejector could not be determined with any certainty. A purely diagrammatical drawing of this region is given in fig. 4. The duct connecting this portion of the ovejector to the uterus, and corresponding in position to the *pars haustrix* of Trichostrongyles with a double uterus, has a thick wall, and was found to be coiled in all the specimens examined.

The uterus contains from six to eight eggs in the larger specimens while only two or three are found in the smaller worms. The eggs are segmented when laid and have an average measurement of 0·07 mm. in length and 0·038 mm. in width. The ovary in this species is somewhat unique in that it extends anteriorly well beyond the junction of the

œsophagus and the intestine in the greater number of the worms examined. In others, and particularly in those containing the smaller number of eggs, the ovary does not in all cases reach the level of the base of the œsophagus.

In fully matured worms the transverse markings which indicate the division between the developing eggs are seen very clearly.



Viannaia talpæ n. sp.

Fig. 1.—Anterior end under low magnification.

Fig 2.—Male bursa in lateral view showing disposition of bursal rays.

Fig. 3.—Spicules in dorsal view.

Fig. 4.—Posterior end of female showing ovejector apparatus and position of anus.

The upper scale applies to Figs. 2 and 3, and the lower one to Figs. 1 and 4.

Male.—There is very little difference in length between the male and female of this species. Measurements were obtained ranging from 1·75 mm. to 2·25 mm. giving an average of 2·1 mm. with a width of 0·05 mm. This latter measurement is slightly less than that given for the greatest width of the female. Other measurements, viz.: length of œsophagus, position of excretory pore from head end and length of the cuticular inflation are closely similar to those given in the case of the female.

The bursa has two equal lateral lobes and no dorsal lobe. When spread out, the bursal rays, with the exception of the dorsal and the externo-dorsal, are seen to extend to the edge of the bursal membrane. The ventro-ventral and latero-ventral rays are joined for a short distance at the base; they are well separated distally and directed forwards. The externo-lateral is entirely separated while the other two laterals are united for a short distance proximally. Both the externo-dorsal and the dorsal rays are fairly long and somewhat slender and arise from a short common trunk. The dorsal ray is bifurcated distally. There is a pair of slender preanal papillæ present.

The spicules are rather indistinct and comparatively short; both are equal in length and measure 0·08 mm. They are almost straight with the anterior end club-shaped and swollen to a thickness about twice that of the shaft. Towards the tip they become split into two prongs for about a third of their length; the larger prong has four short prominences on its lateral side close to the tip. There appears to be no gubernaculum present.

Discussion.

On general characters the species described above falls within the genus *Viannaia* of the sub-family *Heligmosominæ*, and it is proposed to name this worm *Viannaia talpæ* with *Talpa europæa* as its host. It is true that the presence of longitudinal ridges separates this species from other members of the genus *Viannaia* but it can hardly be placed in a new genus on this character alone. One might, on this character, place the species in the closely related genus *Viannella* which contains at least one species with longitudinal ridges namely *Viannella viscaciæ* Goodey, 1925. On the other hand the arrangement of the bursal rays shows a greater

similarity to that found in members of the genus *Viannaia*. As Goodey (1925) points out the differences between these two genera are very slender and it seems questionable whether *Viannella* should be regarded as a valid genus.

Von Linstow (1882) described and figured a male Trichostrongyle from the mole which he considered to be identical with *Strongylus minutus* Duj., 1845, from *Microtus subterraneus*. Both Hall (1916) and Travassos (1921) concluded that these two species are not identical owing to the difference in their size, and in the hosts in which they were found. Travassos tentatively places von Linstow's species in the genus *Viannaia* under the new name *V. linstowi*. The writer is in agreement with this conclusion to separate these species, but finds it impossible to identify *V. talpæ* with *V. linstowi*, although both are found in the same host. According to the figure given by von Linstow, there is undoubtedly some similarity between the two species in the arrangement of the bursal rays, but the spicules, on the other hand, are entirely different; those in *V. talpæ* are comparatively short while in *V. linstowi* they are long and filiform. If these two species, therefore, are to be considered identical, one must assume that von Linstow was in error with regard to the character and the length of the spicules. On the other hand, should his description prove to be correct, his species could not remain in the genus *Viannaia*. Its position in the *Heligmosominæ* must also remain doubtful so long as the female is unknown.

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Two New Genera of Trematodes from a Red-Bellied Water Snake.

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(*From the Department of Helminthology, London School of Hygiene and Tropical Medicine*).

The material upon which this study is based was obtained from a Red-Bellied Water Snake, *Francia abacura*, from Florida, which had died at the Zoological Gardens, London. This particular snake had been at the Gardens for only a fortnight before its death, so unquestionably infestation had taken place in America before transfer to London. No opportunity was afforded the writer for studying the material in a living condition and consequently the measurements were made from preserved material in the Helminthological Collection of the London School of Hygiene and Tropical Medicine.

An examination of the material revealed two distinct genera of trematodes, both of which appear to be new to science. These belong to the widely separated families Plagiorchidæ Lühe (1901) and Halipegidæ Poche (1925).

Acknowledgments.

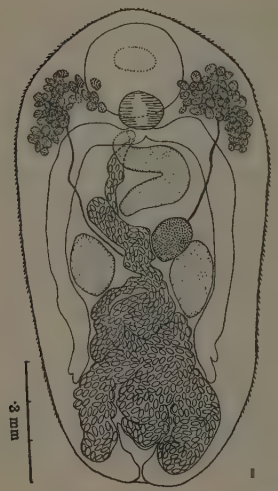
This work was done at the London School of Hygiene and Tropical Medicine at the suggestion of Professor R. T. Leiper, F.R.S., to whom the writer wishes to acknowledge his indebtedness and to express his grateful appreciation for many kindnesses, helpful advice and numerous conveniences placed at his disposal.

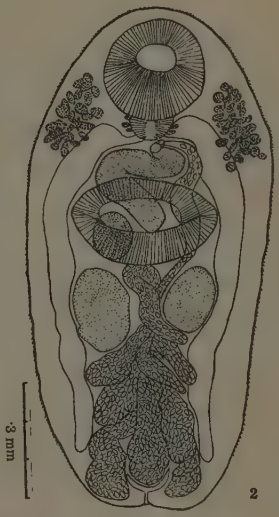
Stomatrema pusilla n.g., n.sp.

(Figures 1 to 8).

Eight specimens of this form were obtained from the mouth of the Red-Bellied Water Snake. Examination showed them to be flattened

dorso-ventrally and to range in length from 1 mm. to 2·3 mm. and from 0·5 mm. to 0·7 mm. in width with the greatest breadth between the oral and ventral suckers. The entire cuticle is thickly beset with spines except at the extreme posterior end where they become somewhat less numerous. The oral sucker is sub-terminal and measures 0·26 mm. in diameter. The ventral sucker has a greater breadth than length, measuring 0·38 mm. by 0·22 mm., and its position in the body is such





Stomatrema pusilla.

Fig. 1.—Dorsal view.

Fig. 2.—Ventral view.

that its posterior edge divides the body length approximately into two equal parts. A globular pharynx, slightly over 0·1 mm. in diameter, is situated immediately behind the oral sucker. In section, a very short prepharynx is discernible but it is not visible in whole mounts. Likewise, in section, a short œsophagus, about 0·03 mm. in length, can be detected. Small pharyngeal glands surround the pharynx which are inconspicuous and are not readily observed except in section. There are also some

gland cells attached to the outer wall of the œsophagus. The œsophagus divides into the two rather conspicuous intestinal cæca which extend nearly to the posterior end of the body.

In the centre of the body the genital organs are situated, dorsal and posterior to the ventral sucker. The oval or spherical ovary, 0·12 mm. to 0·15 mm. in diameter, lies to the right of the median line on the posterior edge of the ventral sucker. From it the oviduct passes medially to a point just beyond the median line where it turns back upon itself.

Fig. 3. Cuticle showing spines of *S. pusilla*.
Fig. 4. Section of pharynx showing pharyngeal and œsophageal glands.

At the point of turning, Laurer's canal is given off which passes to the dorsal surface. The oviduct continues into the oötype surrounded by numerous unicellular gland cells which make up Mehlis' gland. A yolk duct, leading from a yolk reservoir, enters the oötype at the beginning of Mehlis' gland. At the distal end of Mehlis' gland is situated the duct of the large receptaculum seminis. This duct passes posteriorly in a somewhat convoluted tube to the receptaculum which is located between the posterior ends of the testes. The receptaculum seminis measures 0·18 mm. by 0·11 mm. in size but is much flattened dorso-ventrally.

It is not readily observed in whole mounts as it is entirely surrounded by uterine coils laden with eggs. From the juncture of the oötype and the duct of the receptaculum seminis the uterus arises and passes in a winding course to the posterior end where it occupies the area between the testes and intestinal cæca. The uterus passes forward again and terminates in a muscular and glandular metraterm which opens into the genital pore in the median line immediately behind the pharynx.

On either side of the body immediately behind the ventral sucker are situated the testes. The right testis, 0·22 mm. by 0·15 mm., is somewhat larger than the left which is 0·16 mm. by 0·12 mm. in size. Seminal

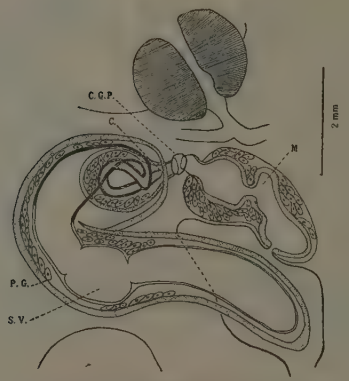


Fig. 5.—*Stomatrema pusilla.* Reconstruction of cirrus-sac and metraterm from dorsal view. Slightly diagrammatic.

C—cirrus ; C.G.P.—common genital pore ; M—Metraterm ; P.G.—prostate gland ; S.V.—seminal vesicle.

tubules connect the testes with a large S-shaped cirrus pouch. The latter is from 0·33 mm. to 0·4 mm. in length by 0·07 mm. in width and encloses a very large seminal vesicle, prostate gland cells and a protrusible cirrus. This organ opens into the genital pore just behind the pharynx. On either side of the pharynx the follicular vitelline glands are confined to

small areas, extending anteriorly as far as the oral opening and terminating posteriorly at some distance in front of the ventral sucker. Vitelline ducts connect the vitellaria with the vitelline reservoir in the median line

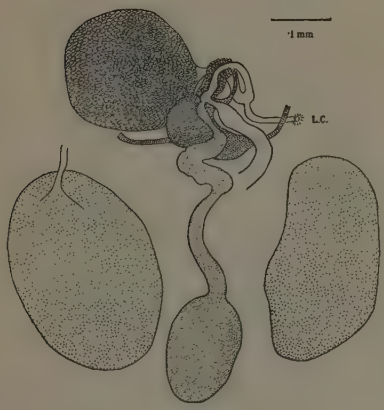


Fig. 6.—Reconstruction of ovary, oötype, receptaculum seminis and testes, ventral view. *L.C.—Laurer's canal.*

Fig. 7.—Eggs of *S. pusilla.*

directly behind the ovary. The numerous mature eggs contain miracidia and have thick, yellowish brown, shells which are provided with opercula. They measure 0·033 mm. to 0·035 mm. by 0·015 mm. to 0·017 mm.

The excretory vesicle is Y-shaped, the stem being entirely covered by the folds of the uterus. Two lateral branches arise from the stem in the posterior region of the testes and extend forward to either side of the pharynx.

This interesting form shows some striking similarities to certain other genera in regard to particular organs or characters. A strong resemblance

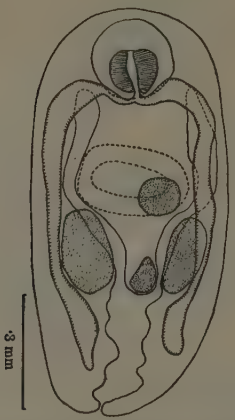


Fig. 8.—*Stomatrema pusilla*; reconstruction showing excretory vesicle.

is noted to *Lechriorchis primus* Stafford and *Zeugorchis æquatus* Stafford (1905 : 691) by the presence of small pharyngeal glands and to *Holometra exigua* (Mühling) as shown by Looss (1899 : 678) relative to the arrangement of the vitellaria. However, there are certain marked differences between their reproductive organs which distinctly separate them into different genera. The type and arrangement of the receptaculum seminis is very similar to that displayed in *Pleurogenes gastroporus* Lühe (1901 : 166) but there is a marked contrast in the position of the genital pore and ovary. The above marked characteristics and the Y-shaped excretory vesicle, the large ventral sucker, the size, shape and type of the cirrus-sac,

and the position of the genital pore seem sufficiently distinctive to designate this form as a new genus and species.

Genus and Type Species :—*Stomatrema pusilla.*
Host :—Red-Bellied Water Snake, *Francia abacura.*
Habitat :—Mouth.
Locality :—Florida.

Vitellotrema fusipora n.g., n.sp.
(Figures 9 to 13).

The description of this genus is based upon three specimens taken from the stomach of the Red-Bellied Water Snake. They measure 2 mm., 2·5 mm. and 3·5 mm., respectively in length, by 0·7 mm. to 1 mm. in breadth. The body is round in cross section with the thickest part in the region of the ventral sucker and tapering towards the ends : the decrease in diameter being greater at the posterior end. There are no spines in the cuticle which is relatively smooth. The sub-terminal oral sucker is 0·35 mm. in diameter while the ventral sucker measures 0·6 mm., the latter being situated in the middle of the body. Behind the oral sucker is a slightly elongate pharynx, 0·14 mm. in diameter. There is no prepharynx but a short œsophagus is present. The œsophagus divides into prominent cæca which extend to the extreme posterior end.

Some striking peculiarities occur in the arrangement and position of the reproductive organs. The testes are lateral and are situated directly behind the ventral sucker, one in either side of the body. The right testis, 0·3 mm. by 0·22 mm. is slightly larger than the left, which measures 0·16 mm. by 0·2 mm. in size. Seminal ducts from the testes unite dorsal to the ventral sucker and continue as the vas deferens to the seminal vesicle, a short distance behind the pharynx. The seminal vesicle is a somewhat elongate, thick-walled organ measuring from 0·12 mm. to 0·18 mm. by 0·12 mm. in size. An ejaculatory duct leads from it to the genital pore through a muscular and glandular organ resembling a cirrus pouch. Undoubtedly, the gland cells are prostatic in function. There is no cirrus.

In shape the ovary is slightly oval and is 0·1 mm. to 0·12 mm. in diameter. It is situated in the posterior end of the body between or behind the ends of the cæca. In two of the three specimens the ovary

occurs in front of the yolk glands while in the third it is behind as shown in the accompanying figures. This discrepancy may be due either to

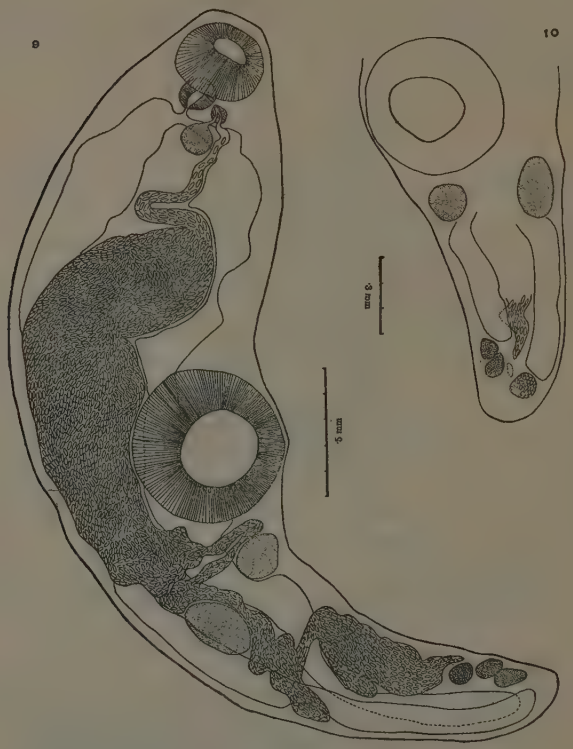


Fig. 9.—*Vitellotrema fusipora*, ventral view.

Fig. 10.—*V. fusipora*, posterior end showing variation in position of ovary and vitellaria, dorsal view.

a distortion of the worms through contraction during preservation, or possibly it may be a variable character in the arrangement of this organ. A somewhat similar peculiarity is recorded by Stafford (1905 : 688) in *Halipegus occidualis*. On the posterior (or anterior) edge of

the ovary the oviduct arises and almost immediately curves dorsally whence it gives off Laurer's canal. This latter organ proceeds dorsally in a winding course and opens on the dorsal surface. The oviduct turns ventrally from the junction of Laurer's canal, receives the vitelline duct and becomes the oötype. Here it is surrounded by Mehlis' gland in the median line of the body. The oötype continues into the uterus which proceeds anteriorly in a winding course. When the uterus reaches the middle of the body it enlarges into an enormous organ filled with eggs displacing the other organs in its immediate vicinity. The

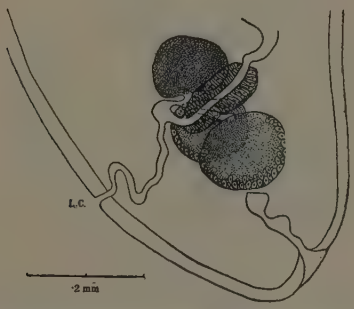


Fig. 11.—Reconstruction showing ovary, oötype and vitellaria from right side. *L.C.—Laurer's canal.*

uterus continues to the region behind the pharynx when its walls become thickened into a vagina. This enters the cirrus pouch and there fuses with the ejaculatory duct from whence the common duct opens into a common atrium before reaching the exterior as the genital pore ventral to the pharynx. This peculiarity of the union of the male and female organs is a striking departure from the usual arrangement found in trematodes. A similar condition is approximated in *Halipegus occidualis* as described by Stafford (1905 : 688), however, in that instance the ductus ejaculatorius and vagina only open into a common atrium but do not fuse before doing so as occurring in the case under observation.

Two oval-shaped yolk glands, 0·15 mm. by 0·13 mm. in size, are located in the posterior end near the ovary. In two specimens they are situated behind and in one they are in front of the ovary. These yolk glands seem to function both as glands and as yolk reservoirs. The periphery of each gland is made up of one or more layers of glandular cells while the inner portion is composed of yolk cells. Ducts from each unite into a common vitelline canal which enters the oviduct in front of Mehlis' gland.

The numerous eggs containing miracidia are thick-shelled and yellowish brown in colour and are provided with a long polar filament

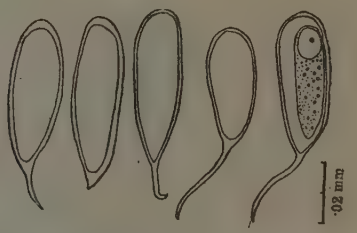


Fig. 12.—Eggs of *Vitellotrema fusipora*.

or spine. They measure 0·042 mm. to 0·054 mm. by 0·015 mm. to 0·017 mm. in size. The polar filament varies from 0·015 mm. to 0·03 mm. in length.

It was impossible to follow out the excretory vesicle but it is apparently of the Y-type with a short stem. Many excretory tubules are clearly visible in section which appear to anastomose and to form a network connected with the short stem.

Vitellotrema fusipora is remarkably singular with respect to some of its characteristics. Perhaps, the most striking peculiarity is the position and the relationship of the paired vitellaria in the posterior end of the body. It shares in this respect, the somewhat similar arrangement

found in *Halipegus* Looss (1899) as shown by Lühe (1909 : 135), *Progonus* (Levinsen 1881) Looss (1899), *Derogenes* Lühe (1909 : 134) and to the genera of the family Hemiuridæ Lühe (1909 : 136). However, there is

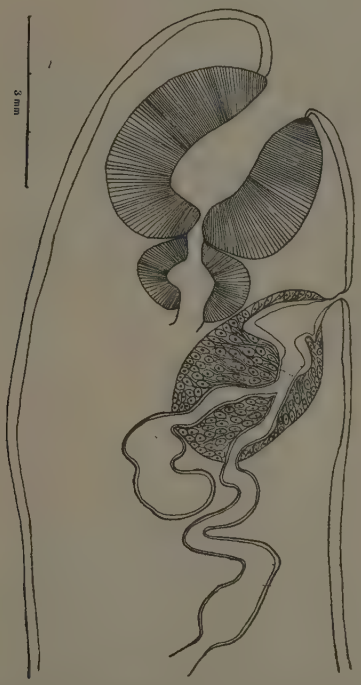


Fig. 13.—Reconstruction of seminal vesicle, vagina, cirrus pouch and genital atrium.

a marked disagreement with *Derogenes* in regard to the position and folds of the uterus, prostate gland and the length of the intestinal cæca while the members of the Hemiuridæ possess the posterior tail-like appendage which is a marked distinction from *Vitellotrema*. There is also a dis-

similarity with *Progonus* because the latter has a different arrangement of the uterus, a large cirrus-sac and its intestinal cæca are connected at their posterior ends. In *Halipegus* the paired vitelline glands are each composed of four or five follicles whereas in *Vitellotrema* each gland is a single spherical or oval body. The eggs of *Halipegus* and *Vitellotrema* are similar in structure and size, the latter being slightly larger but the former have a somewhat longer filament.

The peculiar relationship of the paired vitellaria, the position of the ovary and testes, the arrangement of the uterus, and the fusion of the ductus ejaculatorius and vagina before entering the genital atrium, with the relative positions of the oral and ventral suckers are deemed sufficiently characteristic to establish a new genus and species for this trematode.

Genus and Type Species:—*Vitellotrema fusipora.*
Host:—Red-Bellied Water Snake, *Francia abacura.*
Habitat:—Stomach.
Locality:—Florida.

Classification.

The family Plagiorchidæ Lühe (1901 : 173) has been the subject of much controversy and has been revised a number of times during recent years. Odhner (1911 : 22) established the family Lepodermatidæ but Ward (1917 : 5, 1918 : 402) considered it to be identical with Plagiorchidæ Lühe but accepted Odhner's revision. More recently, Baer (1924 : 26) has again utilized the family Lepodermatidæ and has divided it into three sub-families. However, Poche (1925-6 : 129) takes exception to the classification of Baer and places all of his sub-families, including the Reniferidæ (Pratt) Baer, back into synonymy with the family Plagiorchidæ This seems to the writer to be the more logical procedure as it appears that Baer has insufficient morphological bases for the separation of his many sub-families.

Stomatrema pusilla n.g., n.sp., because of its diagnostic characters, naturally falls into the family Plagiorchidæ, The arrangement of the testes and ovary, the type and position of the cirrus-sac, the type of

excretory vesicle and the particular distribution of the vitellaria leave no question as to its family relationship.

The peculiar morphological characteristics shown by *Vitellotrema* present some difficulties in establishing its family relationship. However, since it shows a closer affinity to the genus *Halipegus* Looss than to any other on account of the position of its ovary and vitellaria it appears that it should be provisionally included in the family Halipegidæ which was erected by Poche (1925 : 198). In this family Poche has placed the single genus *Halipegus* Looss which he has designated as the type for the family. There is some similarity to *Derogenes* Lühe and *Progonus* (Lev.) Looss but the type of uterus and prostate gland in the former and the large cirrus-sac and the posteriorly connected cæca of the latter will not permit their inclusion in the family Halipegidæ. A minor alteration of the diagnostic characters of the family Halipegidæ is necessary to admit the genus *Vitellotrema*. This is in regard to the paired vitellaria which should be modified to include paired entire spherical or oval-shaped vitelline glands as well as paired vitellaria composed of four or five follicles each. With this modification *Vitellotrema* is included with *Halipegus* Looss in the family Halipegidæ Poche.

Habitat :—In mouth, pharynx and stomach of amphibia and reptiles.

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Journal of Helminthology, Vol. VI, *No.* 4, *December*, 1928, *pp.* 219-222.

A New Definitive Host for *Schistosoma mansoni*.

By T. W. M. Cameron, M.A., Ph.D., D.Sc., M.R.C.V.S.

(*Lecturer and Milner Research Fellow in the Department of Helminthology, London School of Hygiene and Tropical Medicine.*)

Until now *Schistosoma mansoni* has been regarded as an essentially human parasite. In 1859, Cobbold recorded from *Cercocebus fuliginosus* a species of Schistosome which he called *Bilharzia magna*. The only specimen which has been preserved was found to be a fragment of a male by Leiper (1915), who states, " I have been quite unable to identify it with either of the species now recognised in man." Various authorities have, however, referred this species to *S. hæmatobium*. *S. mansoni* has been grown experimentally by Leiper in rats, mice, guinea pigs and African and Indian monkeys, and by Lutz in rabbits. It has never been found naturally in any animal other than man.

On a recent visit to the West Indies, I have found it in monkeys in the Island of St. Kitts. This island is one of the most northern of the Lesser Antilles and is now part of the Colony of the Leeward Islands. It is purely volcanic in formation and consists of two groups of mountains in close proximity to each other and separated from a third or minor group in the south by means of a narrow, low lying isthmus ; this southern portion is virtually uninhabited. It has few permanent streams, most of the rain water being carried to the sea by " guts " or river beds, normally dry except during the rainy season. The permanent streams are two in number—Wingfield River, entering the sea at the village of Old Road on the leeward side of the island and originating in the northern group of mountains, and Cayon River, which originates in the central of the three mountain groups, and enters the sea at Cayon village. In addition, there is a third stream of a semi-permanent nature, which during flood time reaches the sea at West Farm village, near Old Road,

but which in the dry season only supplies sufficient water (by means of a dam some way up the hills) for a piped supply to the plantation of West Farm itself. There was a fourth stream north of this at one time, but a dam high up in the mountains diverts the supply to the town of Basseterre, and the empty bed now only functions as a " gut."

Like so many of the other West Indian islands, the local mammalian fauna is an entirely imported one, and in addition to the usual domestic mammals, consists of mongoose and a species of African monkey, introduced many years ago and recently identified by Dr. G. M. Vevers, Superintendent of the London Zoological Gardens, as *Cercopithecus sabæus* (the West African green monkey). The mongoose had been introduced for the purpcse of keeping down snakes and rats. There are now few or no snakes in the island, but the mongoose has been unsuccessful in exterminating rats. It has, however, turned its attention to domestic fowls and rendered chicken-keeping an almost impossible venture. A considerable number of specimens examined shewed no presence of helminthic parasites at all.

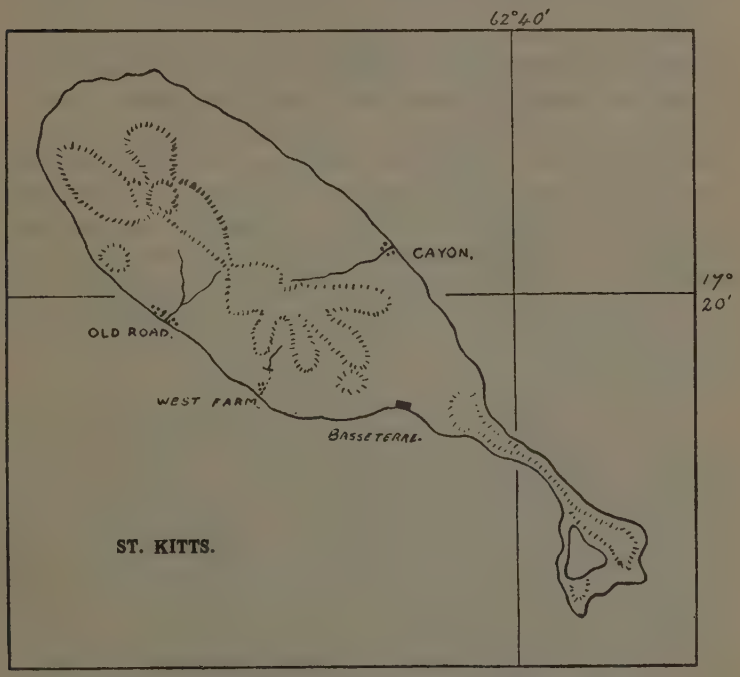
The monkeys were introduced as pets, but during the French wars they escaped to the mountains, and settling down multiplied alarmingly, and seriously interfered, not only with sugar cane planting, but with fruit and vegetable culture. These monkeys live in packs, each of which appears to have a definite area, which it seldom leaves.

Schistosoma mansoni has been know to occur in the Leeward Islands since the parasite was first recorded by Manson in 1902. Manson's case came from Antigua, but there is reason to suppose that the disease may have been contracted in St. Kitts. In 1918, Dr. S. B. Jones drew attention to the prevalence of Bilharziasis in St. Kitts, and a recent survey by the writer has shewn that, while a considerable number of people are infected with the parasite, it is practically confined to the two areas wherein lie the permanent streams, viz., the Old Road—West Farm area and the Cayon area.

An examination of a number of monkeys from various parts of the island was made, and of seven received from these two areas, five were found to be infected with *Schistosoma mansoni*. Of these, four had light infections, while one was suffering from a severe dysentery due to the large number of parasites in the walls of both the small and the large intestines.

Monkeys from the "dry" parts of the islands, where there is no natural permanent water supply, were invariably free from the flukes.

The same species of monkey is present in Nevis, Grenada and Barbados; but *Schistosoma mansoni* is apparently absent from all these islands. In Trinidad various species of South American monkeys occur, but no



Sketch map of St. Kitts, shewing the areas where endemic human and simian bilharziasis occurs.

African species. Monkeys are absent from the other islands in the Lesser Antilles.

Eradication of human Bilharziasis is a problem of considerable importance, not only in the New World, but in Africa. Two principal plans of eradication have been suggested, based respectively on eradication of the molluscan intermediary, or on the destruction of the parasite in

the definitive host by Antimony. If the parasite were exclusively human, as had hitherto been believed, the latter plan would have, at least theoretically, a certain chance of success. Where, however, it is found to occur in wild animals, this plan, to be successful, postulates the entire destruction of these reservoir hosts or, alternatively, its adoption in isolated areas where these reservoir hosts are absent and can be kept absent. Leiper has already shown that rodents, as well as monkeys, can be readily infected experimentally, and this discovery of the parasite naturally occurring in African monkeys—even in the New World—suggests the possibility of other reservoir hosts which might be equally difficult to eradicate. The first plan—eradication of the intermediate host—offers no such obstacles, and in the presence of an undomesticated reservoir host, seems to be the only one which offers hope of permanent result. So long as wild animals harbour the parasite, a potential source of infection of the snails must remain. Filtration or storage of water and similar measures aiming at the destruction of the cercariæ can, at best, be purely palliative ; while removal of the chances of contamination of the water supplies by human fæces can only lessen, not destroy, the molluscan infection.

In St. Kitts, the piped water supply to Basseterre and many of the villages is drawn high up in the mountains from streams which appear to be free from Planorbis. The drinking water at Cayon is likewise piped from a mountain spring free from snails ; but the drinking water to Old Road and West Farm, together with the washing water in all the endemic areas, is not free. In the case of an island such as this, eradication of all the monkeys by concerted government action is a possible, if not very practicable or economic, course, which combined with a simultaneous treatment of all persons harbouring the parasite, might eliminate the infection. Elsewhere, however, even this method is not available, and snail eradication, in St. Kitts, as elsewhere, seems the most promising solution.

Journal of Helminthology, Vol. VI, *No.* 4, *December*, 1928, *pp.* 223-226.

A Cryptic Infection with *Dibothriocephalus latus.*

By R. T. Leiper, M.D., D.Sc., F.R.S.

(*Director of the Division of Medical Zoology, London School of Hygiene and Tropical Medicine.*)

An anæmia, resembling pernicious anæmia, has been commonly associated with *Dibothriocephalus latus* infection in man, but the experimental evidence in support of clinical observation has not yet been forthcoming.

In the Journal of Helminthology, vol. II., pp. 151-166, Miss G. Z. L. Le Bas (1924) recorded observations on three experimental infections in man acquired by swallowing plerocercoids obtained from pike, *Esox lucius*, caught in Lake Neuchatel.

In two of the cases the infection was maintained for 106 days, and treatment with liquid extract of Male Fern and preceded and followed by magnesium sulphate. Up to the time of treatment no appreciable anæmia was produced, nor was there a definite diminution of erythrocytes. Leucocyctosis coupled with an increase of polymorphonuclear cells was observable in a slight degree. Eosinophilia increased to about 15-16 per cent. about three weeks after infection and thereafter gradually diminished to about 2 per cent.

Drs. R. D. Passey and J. Carter Braine measured the red cells in the experimental cases and plotted Price Jones curves to show the distribution of the cells. They found that "there was a small, but definite, increase in the average size of the red cells."

From her observations Miss G. Z. L. Le Bas concluded that "too great a prominence is accorded to the pathogenic powers of *Dibothriocephalus latus* which in normally healthy persons need not produce any lasting untoward effects."

In the record of case A, who had swallowed four plerocercoids, Miss Le Bas stated that the worms obtained by treatment were very broken and no accurate measurements could be obtained and the heads were not found, but that repeated negative examinations of the stools for ova showed, however, that the treatment had been entirely successful.

The purpose of the present note is to record the later history of this case A, which had been treated on 11th January, 1923.

In February, 1928, the patient (R. T. L.) contracted in Egypt, during a short stay there, an attack of dysentery due to Flexner's bacillus. On the morning of the 8th February, 1928, in connection with this infection, while examining his stool microscopically, he discovered large numbers of eggs of *Dibothriocephalus latus*. These have continued to appear in varying numbers up to the present time (December, 1928).

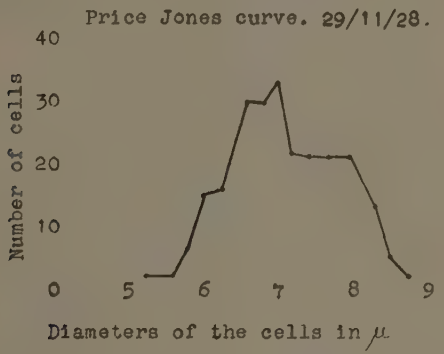
There seems very little doubt but that the present infection of *D. latus* is a continuation of the experimental infection acquired on 27th September, 1922, and treated on 11th January, 1923, for a recent infection would have shown a marked increase in eosinophilia as in the earlier infection. At the time the infection was rediscovered, however, the eosinophil count kindly made by Col. Marrian Perry, O.B.E., was less than 2 per cent. At a later count (on 1st May, 1928) the average of 500 cells gave Polys. 55·4 per cent., Monos. 15·8 per cent., Lymphs. 26·8 per cent., Eosinos. 2·0 per cent., while the total count gave Red cells 3,647,500, and White cells 6,125.

Dr. Kerr Blackie, Demonstrator and Research Student in the Department of Tropical Pathology of the London School of Hygiene and Tropical Medicine, kindly made a fresh investigation of the blood on November 29th, 1928. He reports as follows:—"The blood showed red cells 4,810,000 per cmm., Hæmoglobin 85 per cent. Colour Index 0·89, white cells 6,200 per cmm., with a differential count as follows: Polymorphonuclears 56·65 per cent., Large Mononuclears 16·7 per cent., Lymphocytes 25·65 per cent., Eosinophiles 1·0 per cent. The red cells presented no irregularity of size, shape or staining reaction while the white cells showed a relative mononuclears increase—mainly on the part of the Large Mononuclears. No abnormal cells were found.

"A Price Jones curve indicated a variation in the size of red cells between 5·25μ and 8·75μ with a mean diameter of 7·1μ. 250 red cells were plotted."

The clinical symptoms attributable to the infection are tenderness occasionally in the epigastrium, beneath the gall bladder, and at other times in the region of the umbelicus and the appendix.

These periods are probably associated with periodic sexual activity of the worm, or are due to a change in the location of the head. The early attacks of diarrhœa have entirely disappeared, but at times the latter part of the stool is unformed.



It is to be noted that the abdominal distress recorded by the patient has persisted, although it was stated in Miss Le Bas's paper that they should not be definitely attributed to the presence of the worm " as they were absent in the two other cases " and had continued " after completely successful treatment for the removal of the worm had been carried out."

The only other features worth recording are the recurrence in this case of the symptom of " feeling of having taken tainted food " which has recurred from time to time since the beginning of the experiment and the evanescent appearance of slight jaundice.

A remarkable feature of the case is the complete absence of the passage of segments. No portion of the worm has been passed since its presence was accidentally discovered ten months ago, and its survival since 1923 without detection is undoubtedly due to this fact.

That no anæmia has developed from the infection after a period of five years bears out Miss Le Bas's early conclusion that there is probably some contributory factor involved in the onset of anæmia in cases of *Dibothriocephalus latus*.

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New Genera.

New Species.